



Le système circadien : cible pharmacologique pour prévenir ou améliorer les symptômes associés au cancer et à ses traitements

Pasquale Fabio Innominato

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par

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**Le système circadien : cible pharmacologique pour prévenir
ou améliorer les symptômes associés au cancer et à ses
traitements**

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SOLES OCCIDERE ET REDIRE POSSUNT;
NOBIS, CUM SEMEL OCCIDIT BREVIS LUX,
NOX EST PERPETUA UNA DORMIENDA.

Gaius Valerius Catullus, 84-54 a.C.

*à SARA et à PHOEBE,
pour leurs heures dérobées*

Résumé de la thèse

L'objectif principal de ma thèse est l'exploration de l'impact clinique d'une perturbation du système circadien chez les patients cancéreux. J'ai démontré une relation robuste entre le rythme d'activité-repos et survie, symptômes et qualité de vie. J'ai mis en évidence et caractérisé la dynamique du système circadien des patients sous chimiothérapie. J'ai montré qu'une bonne tolérance conditionnait l'efficacité de la chronothérapie. Ces résultats me conduisent à proposer de cibler le système circadien pour améliorer les symptômes des patients et l'efficacité des traitements anticancéreux.

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I. RESUME

Le système circadien contrôle de nombreux aspects temporels de la physiologie et du comportement tant chez l'animal de laboratoire que chez l'Homme. Le dysfonctionnement du système circadien se traduit par la survenue d'altérations à différents niveaux d'organisation : coordination centrale, physiologie circadienne, horloges moléculaires, voies de signalisation. En particulier, une disruption circadienne induite par les vols transméridiens ou le travail posté est associée à la survenue de symptômes généraux, tels que fatigue, troubles de l'humeur et perte d'appétit. Ces symptômes, en liaison avec une disruption circadienne, sont aussi fréquemment ressentis par les patients atteints de cancer, comme conséquence de leur maladie tumorale ou de son traitement.

Mon travail de thèse s'inscrit dans le cadre des recherches concernant le rôle du système circadien dans le développement des symptômes liés aux cancers et à ses traitements, dans la progression tumorale et dans la survie des patients. Il a pour perspective l'identification de nouvelles options thérapeutiques. En particulier, les objectifs généraux de la thèse consistent à définir les relations entre symptômes et système circadien des patients cancéreux avant et pendant chimiothérapie, et quantifient l'impact clinique de la disruption circadienne sur la qualité de vie et la survie. Je me suis particulièrement intéressé aux patients atteints de cancer colorectal métastatique, troisième cancer en incidence et mortalité. J'ai estimé la fonction du système circadien du patient par l'enregistrement du rythme d'activité et repos, biomarqueur de la coordination circadienne, en utilisant un actimètre de poignet, à l'aide d'une méthode non invasive indispensable pour les études longitudinales.

J'ai démontré une corrélation significative entre la sévérité de la fatigue et de l'anorexie, évaluées par le patient d'après le questionnaire de qualité de vie EORTC QLQ-C30, et le degré de disruption du système circadien, chez un total de 252 patients avant chimiothérapie [Innominato P.F. et al., *Integrative Cancer Therapies*, 2009]. J'ai aussi montré une association entre disruption circadienne pendant une chimiothérapie, chronomodulée ou non, et fatigue et perte pondérale chez 77 patients [Innominato P.F. et al., *soumis*]. Ces études situent le rôle du système circadien dans l'apparition des symptômes généraux des patients cancéreux, en l'absence de traitement ou relativement à son administration.

J'ai ensuite montré que la conséquence clinique d'une disruption circadienne ne se limitait pas seulement à ces symptômes généraux, mais comprenait aussi une perturbation de la qualité de vie globale des patients, et de ses dimensions physique et sociales, chez 96 patients [Innominato P.F. et al., *Cancer Research*, 2009]. J'ai démontré la valeur pronostique du rythme circadien sur la survie globale chez 130 patients, dans ce même article, ainsi que la dimension sociale de la qualité de vie chez 443 patients [Efficace F. et al., *Journal of Clinical Oncology*, 2008], tous évalués avant le début d'une chimiothérapie, chronomodulée ou non. J'ai également retrouvé cette association entre perturbation du système circadien et survie chez 77 patients sous traitement [Innominato P.F. et al., *soumis*]. L'analyse de chaque série temporelle de 130 patients nous a permis aussi d'identifier pour la première fois un critère quantitatif de disruption circadienne, un index de dichotomie I<O inférieur à 97.5%. La pertinence clinique de cet index et de cette valeur seuil serviront de base à la réalisation d'études translationnelles ou interventionnelles futures [Innominato P.F. et al., *Cancer Research*, 2009].

J'ai ensuite montré la faisabilité de l'enregistrement simultané du rythme d'activité/repos et du rythme thermique cutané superficiel dans une étude pilote chez 5 sujets sains et 4 patients, qui se poursuit actuellement en collaboration avec le NIH aux Etats Unis. J'ai ainsi constaté une perturbation transitoire et d'intensité variable du système circadien, à travers l'enregistrement continu du rythme d'activité/repos et de la température pendant l'administration du traitement [Scully C. G. et al., *Interface Focus*, 2011].

J'ai enfin cherché à identifier les facteurs influençant l'efficacité de la chimiothérapie chronomodulée, dans l'optique de son optimisation thérapeutique. Avec mes collègues de l'Unité et du Chronotherapy Group, j'ai ainsi démontré que le sexe était un facteur prédictif indépendant du meilleur schéma d'association d'oxaliplatine, 5-fluorouracile et acide folinique, dans la première méta-analyse sur données individuelles de chonothérapie, incluant 842 patients enregistrés dans 3 essais randomisés de phase III [Giacchetti S. et al., *soumis*]. J'ai ensuite mis en évidence une nouvelle liaison entre tolérance et efficacité du traitement, spécifique de la chronothérapie dans un essai clinique randomisé et contrôlé de phase III portant sur 564 patients. Pour la chimiothérapie conventionnelle, j'ai confirmé que la survie des patients présentant une neutropénie chimio-induite était meilleure que celle des patients non neutropéniques. Je n'ai retrouvé aucun effet pronostique des toxicités associées à la disruption circadienne [Innominato P.F. et

al., *Chronobiology International*, 2011 ; manuscrit soumis]. Dans ces deux manuscrits, j'ai mis en évidence pour la première fois une relation inverse entre toxicité et efficacité pour la chronothérapie, avec une meilleure survie chez les patients présentant une meilleure tolérance, et notamment une moindre neutropénie, une moindre fatigue et une moindre perte pondérale.

L'ensemble de ces résultats argumente l'hypothèse que la perturbation du système circadien, avant ou pendant la chimiothérapie, favorise la survenue des symptômes généraux, détériore la qualité de vie et amoindrit l'efficacité et la tolérance de la chronothérapie.

En conclusion, ces travaux me conduisent à proposer une approche thérapeutique innovante visant à protéger et/ou restaurer l'intégrité du système circadien. Cette nouvelle stratégie améliorerait l'index thérapeutique de la chimiothérapie en augmentant son efficacité et diminuant sa toxicité, tout en minorant la survenue de symptômes, en préservant la qualité de vie et en augmentant la survie des patients atteints de cancer. La mise en œuvre d'une telle stratégie s'appuie sur l'enregistrement non invasif de biomarqueurs du système circadien et la personnalisation de l'administration chronothérapeutique.

II. INTRODUCTION

II.1. Principes Généraux de la Chronobiologie

Comment savoir si quelqu'un est vivant ou mort ? La réponse la plus probable est définie d'après la présence ou l'absence d'un rythme, cardiaque ou cérébral.

Pourtant, bien que la biologie, étudie les phénomènes ubiquitaires et fondamentaux de la vie, les aspects temporels des fonctions biologiques sont trop souvent négligés. En fait la vie est rythmique, tant celle de l'Homme que celle des multiples organismes qui peuplent la Terre. La chronobiologie est la discipline de la Biologie qui étudie les caractéristiques temporelles de la physiologie et des fonctions cellulaires, leurs mécanismes et, entre autres, leurs implications biomédicales (Koukkari and Sothorn 2006).

Un rythme décrit une variation temporelle qui se répète selon une allure similaire. De nombreux exemples de rythmes biologiques existent dans la nature, avec des fréquences variables selon le type de rythme. Le but de cette thèse n'est pas de les dénombrer tous ; ce travail est centré sur les rythmes dits circadiens dont la période (durée requise pour une répétition complète du cycle) est comprise entre 20

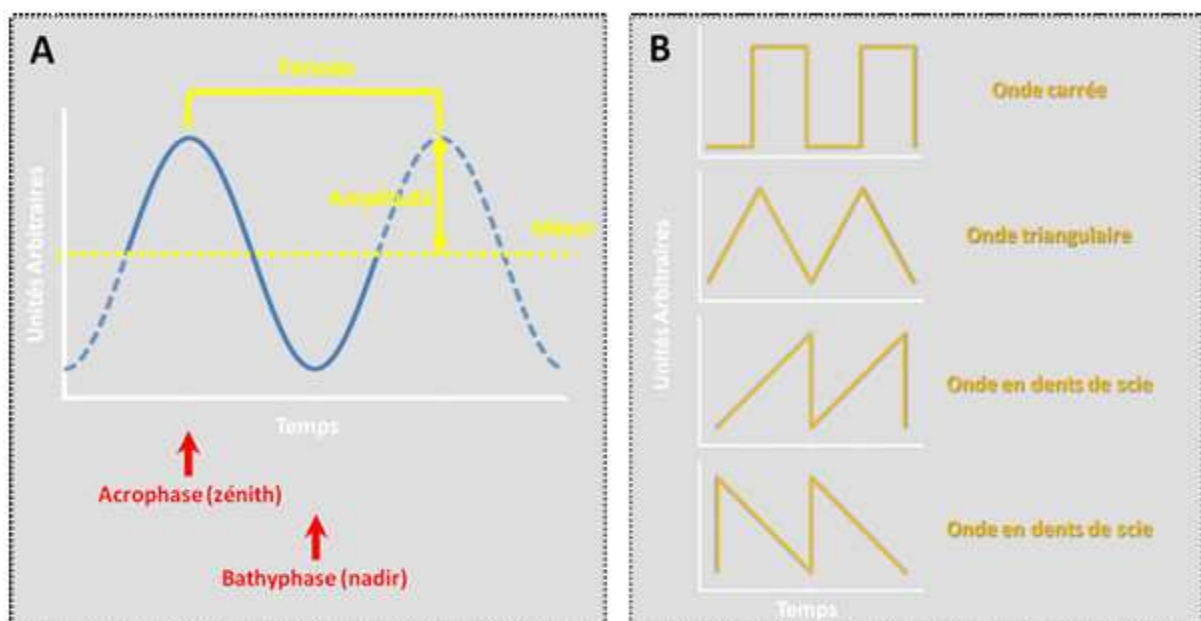


Figure 1. Exemple de modélisation d'un rythme par une fonction sinusoïdale selon la méthode du Cosinor

On peut identifier la période (intervalle de temps mesuré entre deux épisodes qui vont se reproduire identiques à eux-mêmes au cours de la variation), l'amplitude (l'ampleur de l'oscillation d'une onde par rapport à sa valeur moyenne, soit la moitié de la variabilité totale pour une période donnée), le MESOR (Midline Estimating Statistic Of Rhythm, soit la moyenne pour un rythme de période donnée), l'acrophase et la bathyphase (le temps estimé pour atteindre le maximum et le minimum, de la variation par rapport au début d'un cycle).

Tableau I. Glossaire de la terminologie utilisée en chronobiologie.

Adapté de (Koukkari and Sothorn 2006) .

Terme	Définition ou Description
Acrophase	Pic de la courbe mathématique sinusoïdale la plus adaptée aux données. Les unités (temps ou degrés) sont exprimées comme décalage du point de référence.
Amortissement	Diminution de l'amplitude d'un rythme dans le temps.
Amplitude	Distance, pour un modèle mathématique utilisé comme approximation d'un rythme. entre sa moyenne et son maximum
Batyphase	Creux de la courbe mathématique sinusoïdale la plus adaptée aux données. Les unités (temps ou degrés) sont exprimées comme décalage du point de référence.
Cycle	Un profil temporel qui se répète.
Décalage de phase	Changement de l'horaire de la phase d'un rythme.
Entraînement	Couplage de la période et/ou de la phase d'un rythme biologique avec un autre rythme.
Fréquence	Le nombre de cycles pour unité de temps: c'est l'inverse de la période.
Période	Durée d'un cycle rythmique complet, mesuré souvent en heures.
Phase	Emplacement dans le temps d'une valeur particulière du rythme, acrophase par exemple.
Rythme biologique	Changements d'une variable biologique qui se répètent avec un profil similaire et à intervalle systématique, sa période.
Zeitgeber	Signal environnemental périodique ou non qui entraîne et synchronise un rythme biologique.

et 28 heures (h), soit aux alentours d'une rotation terrestre complète, de durée de 23 h 56 min 04 sec (jour sidéral). L'origine étymologique vient de *circa* (environ) et *diem* (jour), c'est-à-dire, de la durée d'environ un jour.

La première description d'un rythme biologique selon l'alternance jour-nuit des feuilles des plantes par Androstène il y a environ 2500 ans, a été suivie par la constatation de la persistance de ce phénomène en obscurité constante, témoin de l'endogénicité des rythmes, par De Mairan au début du XVII^e siècle. Par la suite, la connaissance de la chronobiologie s'est enrichie au cours des siècles de la découverte graduelle des rythmes physiologiques circadiens chez l'Homme (par exemple, de la température rapporté par De Gorter en 1736), de la démonstration de l'horloge centrale chez les rongeurs et chez l'Homme (Stephan and Zucker 1972; Moore and Eichler 1976), et de la découverte de l'horloge circadienne moléculaire (Konopka and Benzer 1971; Roenneberg and Merrow 2005; Lemmer 2009).

Depuis ces travaux pionniers, les applications de la chronobiologie dans le domaine biomédical se sont multipliées, donnant naissance à la chronopharmacologie et à la chronothérapie.

Tous les rythmes, qu'ils soient sinusoïdaux ou de forme plus complexe (en carré, en dent de scie, en triangle, etc.) ont des caractéristiques principales qui sont détaillées dans le Tableau I et dans la Figure 1.

II.2. Le système circadien

II.2.1. Organisation hiérarchique

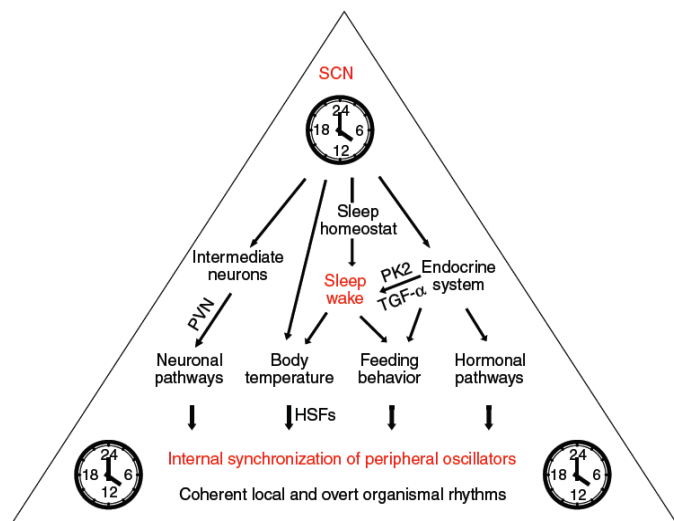


Figure 2. Organisation hiérarchique du système circadien.

Synchronisation interne hiérarchique au niveau de l'organisme. Les noyaux suprachiasmatiques (NSC), l'horloge centrale, sont synchronisés chaque jour par des cycles de lumière-obscurité et par la vie sociale et familiale. Ils coordonnent les phases des oscillateurs périphériques par le biais de multiples voies interdépendantes. Les tissus périphériques peuvent être réglés par les NSC à travers des neurones intermédiaires tels que ceux de la Zone Para-Ventriculaire. Des hormones neuroendocriniennes comme la prokinétine-2 et le transforming growth factor- α régulent directement le cycle de veille et sommeil, qui est ainsi contrôlé par l'interaction entre le NSC et le centre du sommeil hypothalamique (homéostat). Le cycle de veille et sommeil impose ainsi d'autres rythmes circadiens, tels que le rythme d'activité-repos, de prise alimentaire et de la température corporelle. Ces rythmes peuvent ensuite entraîner efficacement les oscillateurs périphériques. Ainsi, l'horloge centrale dans le NSC coordonne les activités des oscillateurs locaux dans les tissus périphériques à travers des rythmes synchroniseurs systémiques et par des voies neuronales, neuroendocriniennes et comportementales, qui, en synergie, contribuent à l'entraînement des oscillateurs périphériques. Cette synchronisation interne est requise pour maintenir les rythmes locaux du corps entier. D'après (Liu, Lewis et al. 2007)

Le système circadien des mammifères est organisé de façon hiérarchique (Figure 2), avec :

- une horloge centrale hypothalamique, les noyaux suprachiasmatiques (NSC) ;
- un ensemble de rythmes physiologiques générés par les NSC ;
- des horloges moléculaires ubiquitaires dans les cellules de tous les tissus périphériques (Reppert and Weaver 2001; Panda, Hogenesch et al. 2002; Reppert and Weaver 2002; Liu, Lewis et al. 2007; Reddy and O'Neill 2010; Zhang and Kay 2010).

Le système circadien est synchronisé par des repères cycliques externes fournis par l'alternance lumière-obscurité, les habitudes

socioprofessionnelles, la prise alimentaire et d'autres facteurs environnementaux. Les rythmes de la physiologie circadienne, tels que ceux de la température ou des sécrétions hormonales, peuvent également servir de biomarqueurs de la fonction du système circadien (Kowalska and Brown 2007; Lévi and Schibler 2007; Liu, Lewis et al. 2007; Ukai and Ueda 2010).

II.2.2. Les noyaux suprachiasmatiques : horloge centrale

L'horloge centrale hypothalamique est constituée d'une paire de noyaux d'environ 20000 neurones, nommée les noyaux suprachiasmatiques (Turek 1985; Moore 1997; Weaver 1998; Welsh, Takahashi et al. 2010). Leur localisation anatomique chez l'Homme est représentée dans la Figure 3. Le rôle central des NSC dans la coordination temporelle des rythmes physiologiques et comportementaux est mis en évidence par au moins trois ordres de preuve :

- La destruction des NSC entraîne la disparition de plusieurs rythmes circadiens tels ceux de l'activité locomotrice, de la température, et de plusieurs sécrétions hormonales (mélatonine et corticostérone ou cortisol), de la fréquence cardiaque et de la prise alimentaire (Stephan and Zucker 1972);
- La transplantation d'une greffe hétérologue de NSC sauvage est capable de restaurer une rythmicité physiologique chez les animaux rendus arythmiques par une mutation génétique ou une lésion des deux NSC; dans tous cas, la période circadienne restaurée est toujours celle du donneur (Ralph, Foster et al. 1990);
- Les neurones du NSC, même isolés, sont les seuls à pouvoir maintenir un rythme circadien *in vitro* pendant plusieurs semaines, en absence de Zeitgeber ou « donneur de temps » (Welsh, Logothetis et al. 1995; Welsh, Takahashi et al. 2010).

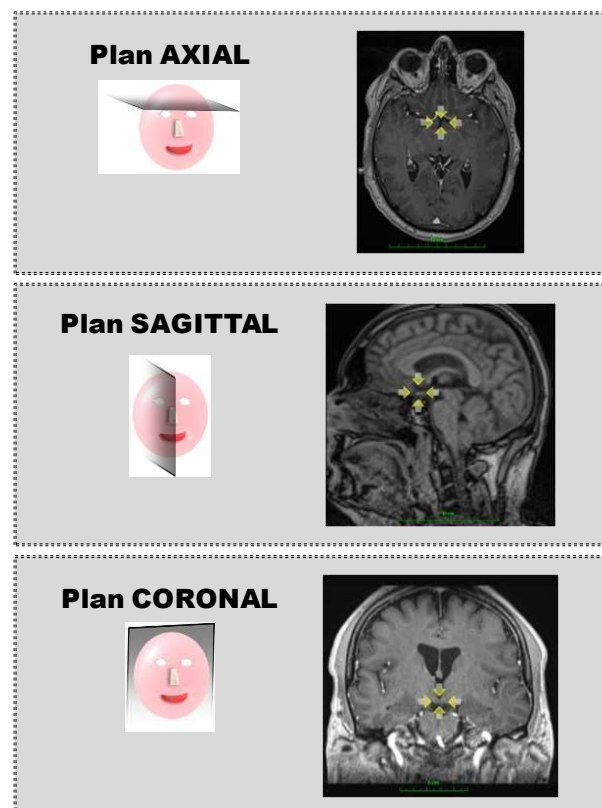


Figure 3. Localisation anatomique des NSC

Localisation anatomique des noyaux suprachiasmatiques (flèches jaunes) chez l'Homme, selon les trois plans spatiaux, axial, sagittal et coronal, sur des images encéphaliques par résonance magnétique nucléaire

II.2.3. L'horloge circadienne moléculaire

La démonstration initiale de l'existence d'une origine génétique de l'horloge circadienne a été fournie chez *Drosophila melanogaster* par Konopka et Benzer en 1971. Ils ont observé et isolé des phénotypes mutants à période courte, à période longue et arythmiques (Konopka and Benzer 1971). Ces phénotypes de l'activité observables en absence de synchroniseurs externes étaient héréditaires et liés au premier gène de l'horloge identifié, nommé *Per* (*Period*) (Konopka and Benzer 1971). Depuis cette découverte historique, les modèles d'horloge moléculaire se sont graduellement complexifiés, par la découverte de plusieurs autres gènes de l'horloge et d'autres facteurs impliqués dans la régulation précise de l'oscillation circadienne et dans la robustesse des rythmes transcriptionnels et translationnels ainsi que dans la réponse

adaptive au fonctionnement des autres systèmes moléculaires

cellulaires. Le modèle le plus récent de l'horloge moléculaire circadienne des mammifères a été décrit et résumé par Zhang et Kay en 2010 (Zhang and Kay 2010) (Figure 4). De façon synthétique, l'oscillateur

moléculaire est fondé sur des boucles de contrôle moléculaire avec une composante positive, qui inclut les gènes *Clock*, *Npas2* et *Bmal1* et une composante négative,

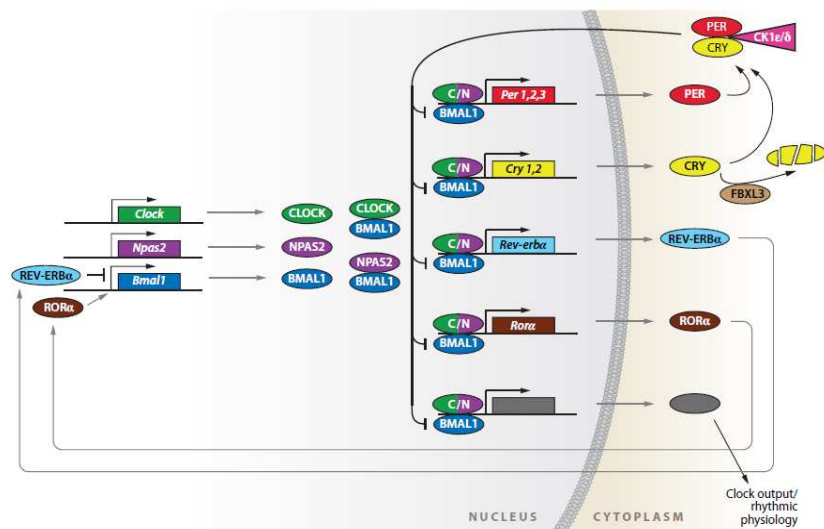


Figure 4. Schéma de l'horloge moléculaire

Modèle simplifié du fonctionnement de l'horloge moléculaire chez les mammifères. L'oscillateur moléculaire est fondé sur des boucles de contrôle moléculaire positif (CLOCK, NPAS2, BMAL1) ou négatif (PER et CRY). Ces boucles sont interconnectées par l'intermédiaire du récepteur nucléaire orphelin REV-ERBa. La transcription des gènes PER et CRY est activée par des hétérodimères formés par BMAL1 (B) et l'une des deux protéines CLOCK (C) ou NPAS2 (N). Les protéines EZH2 ainsi que la caséine kinase 2 (CK2) et SIRT1 (silencing information regulator) interagissent avec ces hétérodimères et facilitent ainsi leur action. L'accumulation et l'activité des protéines PER et CRY sont également influencées par la phosphorylation par les protéines kinases (CK1δ/ε), par l'ubiquitination via un complexe contenant la protéine F-box FBXL3 (spécifique de CRY), par la protéine WDR5 (histone methyl-transférase-binding protein) et par NONO, protéine de liaison à l'ARN et à l'ADN impliquée dans la régulation transcriptionnelle. DEC1 et DEC2 entrent en compétition avec les hétérodimères BMAL1/CLOCK ou BMAL1/NPAS2 pour la liaison à l'E-box et réduisent ainsi la transactivation médiée par cette E-box. Une boucle de rétroaction accessoire, utilisant les récepteurs nucléaires orphelins RORα, RORβ et RORγ comme activateurs, et REV-ERBa et REV-ERBβ comme répresseurs, régule la transcription circadienne de BMAL1. D'après (Paschos, Baggs et al. 2010).

qui inclut les gènes *Per* et *Cry* qui sont interconnectés via une boucle accessoire incluant les récepteurs nucléaires orphelins *Rora*, *Rorβ*, et *Rory* comme activateurs et REV-ERBa et REV-ERBβ comme répresseurs, de la transcription de *Bmal1*. La transcription des gènes *Per* et *Cry* est activée par les hétérodimères protéiques de BMAL1 et CLOCK ou BMAL1 et NPAS2. Les hétéro-quadrimeres entre les protéines PER et CRY inhibent leur propre transcription, générant une oscillation d'environ 24h de leur propre transcription. Plusieurs autres gènes sont impliqués dans la régulation précise de ces oscillations, dont les gènes *Dec1* et *Dec2* dont les protéines interagissent avec les hétérodimères BMAL1::CLOCK/NPAS2 pour l'activation de la transcription de *Per* et *Cry*. Outre ces boucles transcriptionnelles et post-transcriptionnelles, plusieurs modifications post-translationnelles des protéines centrales de l'horloge ont été décrites (Gallego and Virshup 2007; Garbarino-Pico and Green 2007; Ptacek, Jones et al. 2007; Virshup, Eide et al. 2007; Paschos, Baggs et al. 2010) (Tableau II). Les protéines impliquées sont des phosphorylases, des phosphatases, des ubiquitines, des modificateurs des histones et de l'activité de liaison à l'ADN (Figure 4 et Tableau II). De plus, certains microARNs ont été récemment démontrés comme étant des régulateurs importants des rythmes moléculaires de l'horloge circadienne (Cheng, Papp et al. 2007; Pegoraro and Tauber 2008; Hansen, Sakamoto et al. 2011; Kojima, Shingle et al. 2011).

Tableau II. Liste des gènes de l'horloge chez les Mammifères. Adapté de (Zhang and Kay 2010).

Gène	Nom complet	Caractéristiques de la protéine	Fonction pour l'horloge
CORE LOOP CLOCK GENES			
<i>PER1</i> ; <i>PER2</i> ; <i>PER3</i>	period	Régulateurs de transcription avec domain PAS	Répression de la transcription du E-box
<i>CRY1</i> ; <i>CRY2</i>	cryptochrome	Photorécepteurs, flavoprotéines	Répression de la transcription du E-Box
<i>BMAL1</i>	brain and muscle ARNT-like 1	Facteurs de transcription contenant un motif bHLH-PAS ; histone acétyltransferase	Activation de la transcription du E-Box
<i>CLOCK</i>	circadian locomotor output cycles kaput	Facteurs de transcription contenant un motif bHLH-PAS ; histone acétyltransferase	Activation de la transcription du E-Box
PACEMAKERS - INTERLOCKING LOOPS			
<i>DEC1</i> ; <i>DEC2</i>	differentially expressed in chondrocytes	Facteurs de transcription contenant un motif bHLH	Répression de la transcription par CLOCK-BMAL1
<i>NPAS2</i>	neuronal PAS domain-containing protein 2	Facteur de transcription contenant un motif bHLH-PAS	Paralogue de CLOCK ; se dimerise avec BMAL1 pour activer les gènes avec un E-box
<i>TM</i>	timeless	Activité d'heterodimerisation	Interaction avec les autres protéines de l'horloge
<i>DBP</i>	D-site albumin promoter binding protein	Facteur de transcription contenant un motif RAR leucine-zipper	Activation des gènes de l'horloge avec D-box
<i>TEF</i>	thyrotroph embryonic factor	Facteur de transcription contenant un motif RAR leucine-zipper	Activation des gènes de l'horloge avec D-box
<i>HLF</i>	hepatic leukaemia factor	Facteur de transcription contenant un motif RAR leucine-zipper	Activation des gènes de l'horloge avec D-box
<i>E4BP4</i>	E4 promoter binding protein	Répresseur de transcription	Répression des gènes avec D-box
<i>RORα</i> ; <i>RORβ</i> ; <i>RORγ</i>	retinoid-related orphan receptor	Récepteurs nucléaires	Activation des gènes de l'horloge avec RRE box
<i>NR1D1</i> ; <i>NR1D2</i> (<i>Rev-erbAα</i> ; <i>Rev-erbβ</i>)	nuclear receptor subfamily 1, group D, member 1 & 2	Récepteurs nucléaires	Répression des gènes de l'horloge avec RRE box
<i>WDR5</i>	WD repeat-containing protein 5	Sous-unité de l'histone méthyltransferase	S'associe à PER1 ; Réprime la transcription induite par CLOCK-BMAL1
<i>SIRT1</i>	sirtuin 1	Histone dé-acétylase, classe III	S'associe à PER2 ; Réprime la transcription induite par CLOCK-BMAL1
<i>FBXL3</i>	F-box and leucine-rich repeat protein 3	Protéine F-box de la SFC ubiquitine E3 ligase	Médiation de la dégradation de CRY ; régulation de l'expression de CRY et PER
<i>FBXW11</i>	F-box and WD repeat domain containing 11	Protéine F-box de la SFC ubiquitine E3 ligase	Médiation du renouvellement des protéines de l'horloge
<i>PGC1A</i>	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	Co-activateur de transcription	Modification de la fonction des ROR
<i>NONO</i>	non-POU domain containing, octamer-binding protein	Transducteur du signal	Modification de l'activité de CLOCK-BMAL1
<i>CIPC</i>	CLOCK-interacting protein	Interaction avec CLOCK	Répression de la transcription induite par CLOCK-BMAL1
<i>CSNK1D</i>	casein kinase 1, delta	Caseine kinase	Phosphorylation et déstabilisation de PER
<i>CSNK1E</i>	casein kinase 1, epsilon	Caseine kinase	Phosphorylation et déstabilisation de PER
<i>CSNK2A</i>	casein kinase 2, alpha polypeptide	Caseine kinase	Phosphorylation et déstabilisation de PER
<i>RACK1</i>	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1	Protéine kinase	Phosphorylation de CLOCK-BMAL1
<i>PRKCA</i>	protein kinase C, alpha	Protéine kinase	Phosphorylation de CLOCK-BMAL1
<i>AMPK</i>	protein kinase, AMP-activated, beta 1 non-catalytic subunit	Protéine kinase	Phosphorylation et dégradation de CRY1
<i>GSK3β</i>	Glycogen synthase kinase 3 beta	Protéine kinase	Phosphorylation de REV-ERB-α et régulation de sa stabilité
<i>EZH2</i>	enhancer of zeste homolog 2	Histone-lysine N-méthyltransferase	Interaction avec CLOCK et BMAL1 : méthylation des promoteurs de <i>per1</i> et <i>per2</i>
<i>PP1</i>	pyrophosphatase 1	Protéine phosphatase	Régulation de PER
<i>PP2A</i>	protein phosphatase 2A	Protéine phosphatase	Régulation de PER
<i>PP5</i>	protein phosphatase 5	Protéine phosphatase	Régulation de CK1ε
<i>βTrCP</i>	beta-transducin repeat containing	Protéine F-box de la SFC ubiquitine E3 ligase	Médiation de la dégradation de PER
<i>MLL1</i>	mixed lineage leukemia 1	Histone méthyltransferase	Contrôle de l'activité de CLOCK/BMAL1
INPUT GENES			
<i>OPT4</i>	opsin 4	Mélanopsine	Photorécepteur
<i>VIP</i>	vasoactive intestinal polypeptide	Neuropeptide	Synchroniseur neuronal dans le noyau suprachiasmatique
<i>VIPR2</i>	VIP receptor 2	Récepteur couplé à la protéine G	Synchroniseur neuronal dans le noyau suprachiasmatique
<i>ID2</i>	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	Répresseur de transcription contenant un motif bHLH	Régulation de l'entraînement photique
<i>DEXRAS1</i>	dexamethasone-induced Ras-related protein 1	Protéine G similaire à RAS	Potentialisation de l'entraînement photique et suppression de l'entraînement non-photique
<i>cGKI</i>	protein kinase, cGMP-dependent, type II	Protéine kinase dépendant du cGMP	Médiation de l'entraînement photique à travers la régulation de l'expression de PER1 et PER2
<i>GR (NR3C1)</i>	nuclear receptor subfamily 3, group C, member 1	Récepteur des glucocorticoïdes	Synchronisation de la phase circadienne dans les tissus périphériques
OUTPUT GENES			
<i>TGFA</i>	transforming growth factor, alpha	Cytokine	Régulation de la prolifération cellulaire
<i>EGFR</i>	epidermal growth factor receptor	Récepteur avec activité de tyrosine kinase	Régulation de la prolifération cellulaire
<i>NAMPT</i>	nicotinamide phosphoribosyltransferase	Nicotinamide phosphoribosyltransferase	Enzyme limitant la vitesse de biosynthèse du NAD ⁺
<i>MPG</i>	N-methylpurine-DNA glycosylase	N-méthyladénine DNA glycosylase	Médiation du stress génotoxique
<i>MAOA</i>	monoamine oxidase A	Monoamine oxydase	Médiation de l'influence de l'horloge circadienne sur l'humeur
CLOCK MODIFIERS			
> 300 gènes identifiés			

L'horloge moléculaire interagit avec de nombreux systèmes cellulaires, par le biais de signaux faisant intervenir plusieurs autres protéines (Tableau II) (Cermakian and Sassone-Corsi 2000; Hunt and Sassone-Corsi 2007; Eckel-Mahan and Sassone-Corsi 2009; Sahar and Sassone-Corsi 2009; Asher and Schibler 2011).

Tableau III. Liste des cofacteurs ligands des protéines de l'horloge moléculaire circadienne chez les Mammifères.

Protéine de l'horloge	Cofacteur
CLOCK/NPAS2-BMAL1	NAD
NPAS2	Hème
PER2	Hème
CRY1/2	FAD
REV-ERB α / β	Hème
ROR α	Dérivé du cholestérol

(Tableau II).

En outre, les protéines de l'horloge moléculaire interagissent avec plusieurs autres mécanismes cellulaires et, à travers leur liaison, avec des cofacteurs impliqués, par exemple, dans le métabolisme, comme résumé dans le Tableau III.

II.2.4.

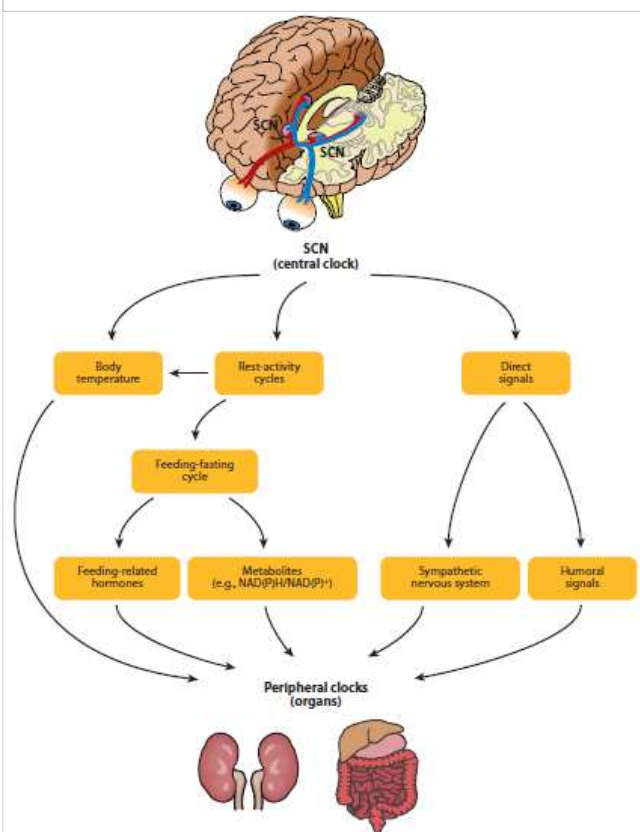
Synchronisation des horloges périphériques

Les noyaux suprachiasmatiques permettent une coordination temporelle à l'échelle de l'organisme grâce à des signaux synchroniseurs pour les horloges moléculaires périphériques.

Les voies d'entraînement impliquées incluent plusieurs rythmes biomarqueurs de la fonction circadienne, tels que l'activité locomotrice, la température corporelle centrale et les sécrétions

En fait, de nombreuses voies de signalisation, impliquées dans presque tous les processus physiologiques cellulaires, ont démontré leur capacité à modifier la phase, l'amplitude et/ou la période de l'horloge moléculaire, selon une étude de criblage par des siARNs ciblant plus de 11 000 produits de transcription (Zhang, Liu et al. 2009)

Figure 5. Voies d'entraînement des horloges périphériques. D'après (Dibner, Schibler et al. 2010).



hormonales, mais aussi des signaux directs via le système nerveux autonome (sympathique et parasympathique), et par le biais de substances paracrines, telles que certaines cytokines, actives au niveau d'autres noyaux du système nerveux central (Buijs and Kalsbeek 2001; Dibner, Schibler et al. 2010). De plus, au niveau cellulaire, l'étroite interaction entre l'horloge moléculaire et les cycles métaboliques de la division cellulaire (Gery and Koeffler 2007; Hunt and Sassone-Corsi 2007; Levi, Filipinski et al. 2007; Eckel-Mahan and Sassone-Corsi 2009; Sahar and Sassone-Corsi 2009) constitue un autre niveau de coordination temporelle périphérique.

II.2.5. Contrôle temporel global de l'expression génique

Le contrôle direct de la transcription génique par les protéines de l'horloge moléculaire, et son couplage à leur fonction de modification de la chromatine, provoquent des oscillations circadiennes de l'expression transcriptionnelle d'environ 10% du génome. Il existe en outre une spécificité tissulaire des gènes dont la transcription est rythmique et dont les mécanismes précis restent à élucider (Delaunay and Laudet 2002; Lowrey and Takahashi 2004; Ueda, Hayashi et al. 2005; Ueda 2007; Ukai-Tadenuma, Kasukawa et al. 2008; Ukai and Ueda 2010; Ptitsyn and Gimple 2011). Comme très peu de gènes contrôlés par l'horloge moléculaire sont communs à divers tissus, certains ont considéré la possibilité que la quasi-totalité des gènes connus au plan fonctionnel soit rythmique au cours des 24 heures. Cette considération a des implications profondes pour l'interprétation des résultats d'études d'expression génomique à partir de prélèvements réalisés à un seul horaire, qui sont de règle en Biologie des Systèmes et en Cancérologie. Elle fournit des bases potentielles pour l'amélioration de l'index thérapeutique des médicaments en optimisant leur horaire d'administration selon l'effet recherché. Toutefois, cet aspect global du contrôle temporel de l'expression génique est encore souvent négligé.

II.2.6. Différences circadiennes liées au sexe

Hommes et femmes diffèrent de façon importante pour plusieurs aspects de leur physiologie et leur comportement. Particulièrement intéressantes sont les différences chronobiologiques liées au sexe qui concernent plusieurs niveaux d'organisation :

- génétique, avec l'existence d'un dimorphisme sexuel des rythmes circadiens des enzymes du métabolisme hépatique des médicaments chez les Rongeurs et du transcriptome de la muqueuse buccale humaine (Krebs, Larkins et al. 2003; Waxman and Celenza 2003; Ahluwalia, Clodfelter et al. 2004; Rinn and Snyder 2005; Clodfelter, Holloway et al. 2006; Bjarnason, Seth et al. 2007; Bjarnason 2010; Hirao, Nishimura et al. 2011) ;
- physiologique, avec, par exemple, la mise en évidence de différences statistiquement significatives selon le sexe de la durée de la période circadienne endogène humaine *ex vivo* (Duffy, Cain et al. 2011), de l'amplitude des rythmes circadiens humains de la mélatonine et de la température (Cain, Dennison et al. 2010), du profil rythmique des concentrations sériques d'IL-6 (Hong, Mills et al. 2005; O'Connor, Motivala et al. 2007; Vgontzas, Pejovic et al. 2007); et de la distribution des chronotypes chez l'Homme (Roenneberg, Daan et al. 2003; Roenneberg, Wirz-Justice et al. 2003; Roenneberg, Kuehnle et al. 2007; Roenneberg and Merrow 2007),
- réponse différente selon le sexe du système circadien à un « stress » ; ceci est illustré par l'existence de différences liées au sexe pour ce qui concerne la suppression de la sécrétion de mélatonine en réponse à une exposition nocturne à la lumière ou pour l'élévation de la sécrétion de cortisol induite par le stress chez l'Homme (Monteleone, Esposito et al. 1995; Burke, Davis et al. 2005) ; il en va de même pour l'induction d'une disruption circadienne par l'irinotécan, un agent anticancéreux chez la Souris (Ahowesso, Piccolo et al. 2009; Ahowesso, Li et al. 2011).

De plus, des rôles direct et indirect ont été décrits pour caractériser les actions des hormones sexuelles mâles (testostérone) et femelles (œstrogènes) dans la modulation du système circadien (Karatsoreos and Silver 2007; Fatehi and Fatehi-Hassanabad 2008; Vida, Hrabovszky et al. 2008; Karatsoreos, Butler et al. 2011). A ces différences circadiennes liées au sexe s'ajoutent celles des cancers et des toxicités de la chimiothérapie liées au sexe, par exemple dans le cancer colorectal ou le cancer bronchique (Milano, Etienne et al. 1992; Yamashita, Mikami et al. 2002; Chansky, Benedetti et al. 2005; Shepherd, Rodrigues Pereira et al. 2005; Singh, Parulekar et al. 2005; Tsao, Sakurada et al. 2005; Press, Zhang et al. 2008; Schwab, Zanger et al. 2008; Rosell, Moran et al. 2009; Mostertz, Stevenson et al. 2010; Cataldo, Gibbons et al. 2011; Cheung, Le et al. 2011). De même, plusieurs études coopératives ont montré que la tolérance au 5-fluorouracile, un antimétabolite largement utilisé pour traiter les cancers digestifs et mammaires, était significativement moindre chez les femmes en comparaison des hommes (Milano, Etienne et al. 1992; Yamashita, Mikami et al. 2002; Chansky, Benedetti et al. 2005; Schwab, Zanger et al. 2008).

II.3. Rythmes biomarqueurs de la physiologie circadienne

II.3.1. Activité et repos

Le rythme circadien de l'activité locomotrice est considéré comme l'un des

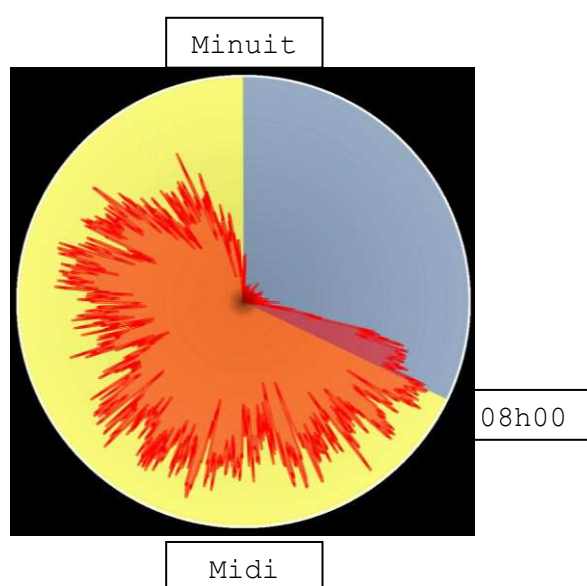


Figure 2. Exemple de rythme circadien d'activité locomotrice chez l'Homme

Exemple de rythme circadien d'activité locomotrice normal chez un sujet sain, enregistré par actimétrie du poignet. Il est ici représenté le nombre de mouvements par minute sur les 24 heures. On note une grande activité pendant la journée (lumière) et une moindre activité pendant la nuit. L'aspect global est plus similaire à une onde carrée qu'à une courbe sinusoïdale.

marqueurs principaux du fonctionnement de l'horloge centrale (Moore-Ede, Czeisler et al. 1983; Moore-Ede, Czeisler et al. 1983; Ortiz-Tudela, Martinez-Nicolas et al. 2010). Ainsi, ce rythme présente des altérations importantes lorsque les NSC sont lésés ou lorsque les gènes de l'horloge circadienne principaux sont mutés chez les rongeurs, en particulier dans des conditions constantes d'environnement (Okamura, Miyake et al. 1999; Zheng, Larkin et al. 1999; Filipski, King et al. 2002; Filipski, Delaunay et al. 2004; Filipski, King et al. 2004; Filipski, Innominato et al. 2005). De plus, la période du rythme d'activité-repos est le reflet précis de la période du rythme circadien de l'activité électrique des neurones isolés du NSC, et ce pour

plusieurs fonds génétiques (Liu, Weaver et al. 1997). Le cycle d'alternance d'activité diurne et de repos nocturne de l'Homme (Figure 6) a été depuis longtemps étudié principalement en psychiatrie et dans l'étude des troubles du sommeil, à l'aide d'un enregistrement continu non invasif, sur un nombre suffisant de cycles, par un accéléromètre porté au poignet : l'actimètre (Figure 7). Son utilisation en oncologie a débuté dans notre Unité en 1998, puis s'est élargie à plusieurs équipes dans le monde. Au 12/06/2011, Pubmed recensait 106 articles avec les mots clés "actigraph*" & "cancer".

Figure 7. L'actimètre

Modèle d'actimètre de poignet (Mini-Motionlogger, Ambulatory-Monitoring Inc., USA). Il s'agit d'un accéléromètre piézo-électrique qui transforme les mouvements sur tous les plans de l'espace en impulsions électriques. Il est muni d'une mémoire pour l'enregistrement des données et d'une interface pour une communication bidirectionnelle avec un ordinateur. Photo de Hlynur Georgsson.



Le rythme circadien d'activité/repos est naturellement très lié aux rythmes qui gouvernent l'alternance veille-sommeil et les prises alimentaires, mais les circuits neuronaux impliqués dans leur génération ne sont pas identiques (Folkard, Wever et al. 1983; Monk, Weitzman et al. 1983; Folkard, Hume et al. 1985; Willie, Chemelli et al. 2001; Saper, Scammell et al. 2005; Brisbane-Roch, Dingemans et al. 2007; Sakurai 2007; Cirelli 2009).

II.3.2. Température centrale

Le rythme circadien de la température corporelle centrale est un biomarqueur de la phase du pacemaker hypothalamique. En effet, la phase et la période de ce rythme semblent refléter fidèlement les variations de l'exposition à la lumière chez l'Homme (Krieger and Hauser 1978; Kronauer, Czeisler et al. 1982; Shanahan and Czeisler

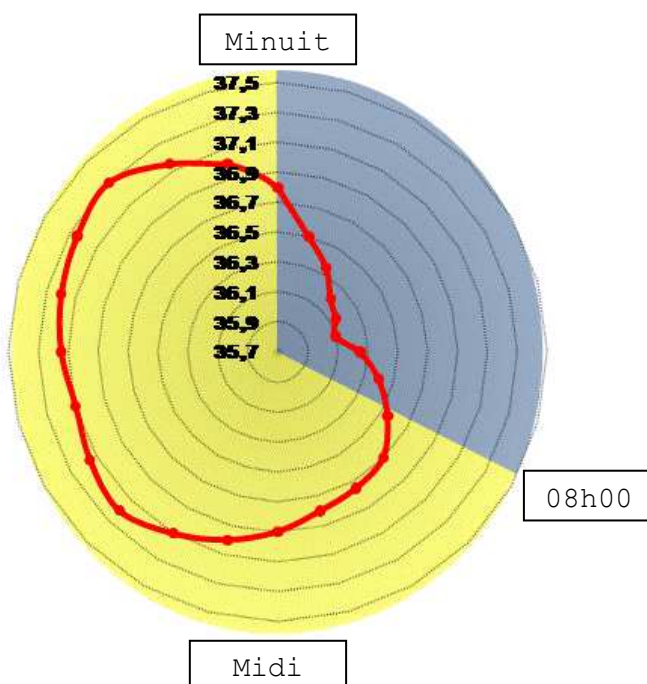


Figure 8. Exemple de rythme circadien de la température corporelle centrale chez l'Homme

Exemple de rythme circadien 'normal' de la température corporelle centrale (en °C), enregistrée par sonde endorectale. On note une baisse de la température centrale au moment de l'endormissement, et une température plus élevée pendant la journée. Redessiné d'après (Kusanagi, Hida et al. 2008).

1991; Martinez-Nicolas, Ortiz-Tudela et al. 2011).

Ce rythme endogène de forme sinusoïdale et de faible amplitude, mais de grande importance clinique, présente physiologiquement un maximum vespéral. Les valeurs thermiques diminuent ensuite progressivement jusqu'au creux nocturne, qui correspond au déclenchement de l'envie de dormir (Van Someren 2000; Van Someren 2006; Lack, Gradisar et al. 2008; Ortiz-Tudela, Martinez-Nicolas et al. 2010) (Figure 8). Le profil du rythme thermique central est inverse de celui de la température superficielle. Ce dernier résulte principalement d'une balance alternée entre tonus sympathique (vasoconstriction) et parasympathique (vasodilatation) des vaisseaux cutanés périphériques, sous contrôle du NSC (Mekjavic and Eiken 2006; Ortiz-Tudela, Martinez-Nicolas et al. 2010). La température cutanée augmente pendant les périodes de repos associés au sommeil et diminue pendant les périodes d'activité en proportion du niveau d'éveil (Van Someren 2000; Van Someren 2006; Lack, Gradisar et al. 2008; Ortiz-Tudela, Martinez-Nicolas et al. 2010).

La température centrale des mammifères représente ainsi non seulement un biomarqueur très important et robuste de l'horloge centrale, mais aussi un synchroniseur universel des horloges périphériques (Brown, Zimbrunn et al. 2002; Buhr, Yoo et al. 2010). Sa mesure clinique présente de ce fait un potentiel très important pour l'évaluation du système circadien. Elle n'est cependant guère aisée à réaliser en raison du manque de dispositifs non ou peu invasifs qui permettent un enregistrement continu pendant un nombre de cycles suffisants pour un calcul adéquat des paramètres statistiques du rythme, notamment de la phase. Les outils actuels possibles incluent :

- une sonde thermique endorectale, difficile à proposer aux patients atteints de maladie sévère à cause de l'inconfort et du risque de perforation,
- une gélule téléométrique à avaler, dont la durée d'enregistrement est fortement conditionnée par la vitesse du transit digestif,
- une mesure cutanée au niveau de l'artère radiale par iButton, potentiellement gênant pour l'utilisation de la main dans la vie courante et facilement influencée par la température environnementale.

II.3.3. Mélatonine

La mélatonine est une hormone synthétisée à partir du tryptophane (Figure 9) et sécrétée par la glande pinéale (ou épiphyse) (Rosenthal 1991; Claustrat, Brun et al. 2005; Lewy 2007). La sécrétion de mélatonine présente un rythme circadien très marqué, avec des concentrations diurnes extrêmement faibles, puis une élévation vespérale avec un pic nocturne (Rosenthal 1991; Moore 1997; Claustrat, Brun et al. 2005) (Figure 10). La phase du rythme et les concentrations circulantes pendant la phase d'obscurité sont influencées par l'exposition à la lumière et contrôlées par les NSC (Lewy, Wehr et al. 1980; Rosenthal 1991; Moore 1997; Claustrat, Brun et al. 2005). La définition du rythme de la sécrétion de la mélatonine nécessite des prélèvements rapprochés

surtout pendant l'obscurité à cause de son profil. Ces prélèvements sont difficiles à réaliser en pratique chez les patients cancéreux non hospitalisés. L'étude de ce rythme demeure cependant importante en raison de son effet afférent sur les NSC, munis des récepteurs à cette hormone (Sprouse 2004). Son action de synchronisation de l'horloge circadienne centrale, et possiblement de certains

Figure 3. Voie de la biosynthèse de la mélatonine à partir du L-tryptophane.

NAT: N-acétyltransférase. D'après (Koch, Nagtegaal et al. 2009).

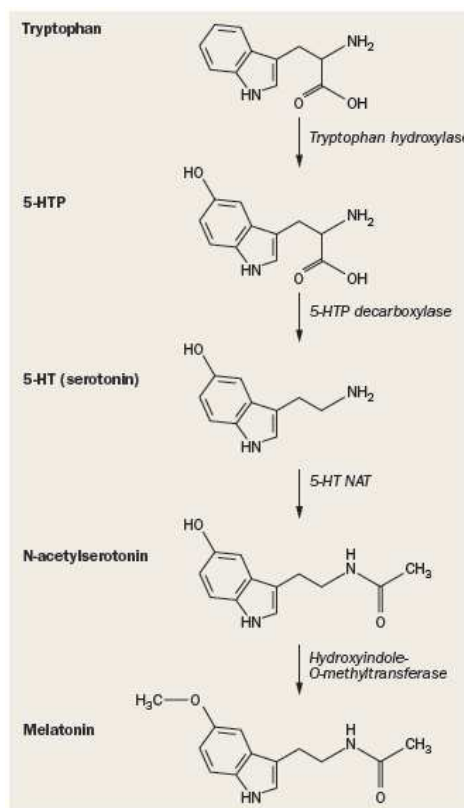
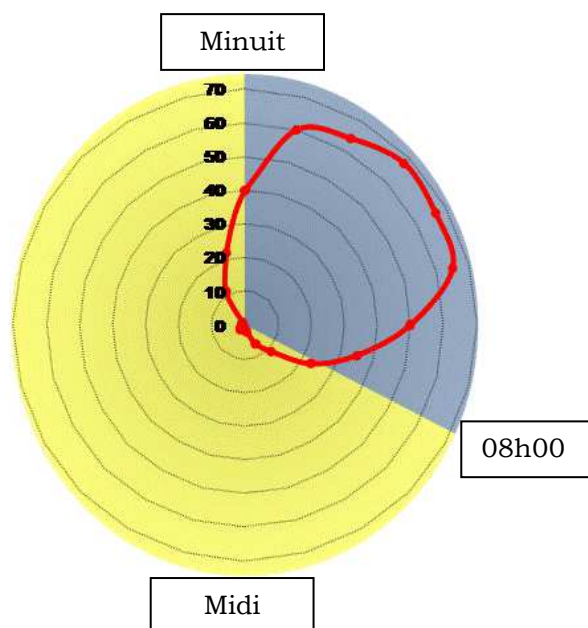


Figure 4. Rythme circadien de la mélatonine sérique (en pg/ml) chez l'Homme.

On note le pic de la mélatonine au milieu de la nuit et des taux proches du zéro pendant la journée (lumière). Les prélèvements pendant la nuit sont effectués avec une lumière de très basse intensité pour ne pas inhiber la sécrétion de la mélatonine. Redessiné d'après (Kusanagi, Hida et al. 2008).



rythmes physiologiques et comportementaux, avec aussi un profil d'innocuité largement démontré, font fréquemment utiliser la mélatonine exogène comme agent chronobiotique (Touitou and Bogdan 2007; Sack 2010; Hickie and Rogers 2011). Enfin, et malgré l'absence de preuves validées de ses effets thérapeutiques, en dehors de certains troubles du sommeil, il existe une abondante littérature scientifique en faveur du rôle de la mélatonine dans les processus cancéreux ou leurs traitements (Cerea, Vaghi et al. 2003; Lissoni, Chilelli et al. 2003; Blask, Dauchy et al. 2005; Lewy, Emens et al. 2006; Jung-Hynes, Reiter et al. 2010; Mediavilla, Sanchez-Barcelo et al. 2010).

II.3.4. Cortisol

Le cortisol est une hormone indispensable à la vie synthétisée à partir du cholestérol et sécrétée par le cortex de la glande surrénalienne (Chung, Son et al. 2011) (Figure 11). Il présente un rythme circadien très ample, avec un pic matinal précoce, suivi par une décroissance diurne progressive. Les concentrations nocturnes sont extrêmement faibles (Koukkari and Sothorn 2006) (Figure 12). Le profil plasmatique des concentrations de cortisol libre correspond bien à celui du cortisol salivaire, notamment pour la période et la phase. De ce fait, l'estimation du rythme du cortisol salivaire paraît mieux adaptée à l'étude de ce biomarqueur chez les patients cancéreux (Koukkari and Sothorn 2006). La sécrétion rythmique de cortisol est la conséquence du contrôle circadien de l'axe hypothalamo-hypophyso-surrénalien par les NSC, et de la

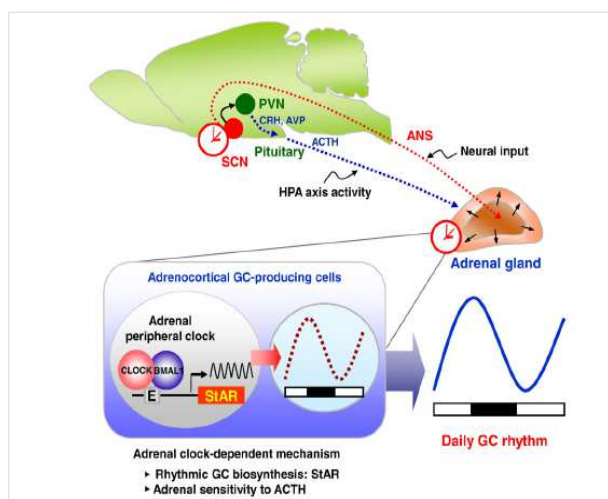


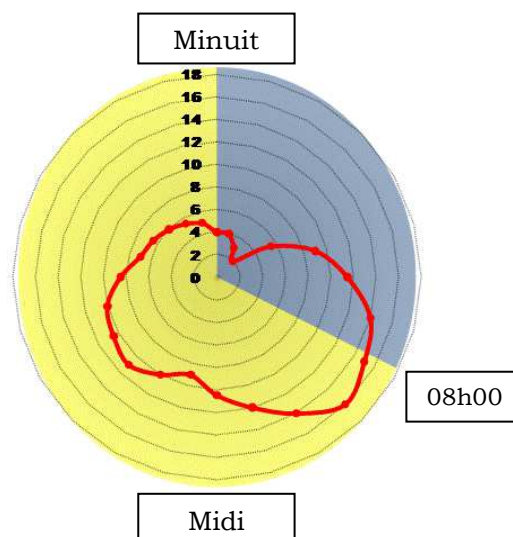
Figure 6. Contrôle moléculaire de la sécrétion du cortisol par la glande surrénalienne.

Modèle de la régulation de la sécrétion diurne et la biosynthèse des glucocorticoïdes surrénaliens. Les variations quotidiennes des taux circulants du cortisol sont réalisés par de multiples mécanismes de régulation, impliquant les noyaux suprachiasmatiques, l'axe hypothalamique-hypophysaire-corticosurrénalien et le système nerveux autonome. En plus de ces mécanismes centraux, les glandes surrénales possèdent d'autres mécanismes intrinsèques impliquant l'horloge circadienne moléculaire. Par exemple, l'expression cyclique de STAR, un gène limitant la vitesse de la biosynthèse des stéroïdes, est directement contrôlé par l'hétérodimère CLOCK:BMAL1 comme un gène contrôlé par l'horloge spécifique à la glande surrénalienne. En plus, dans les tissus périphériques, l'activité transcriptionnelle du récepteur des glucocorticoïdes est modulée par des transformations post-translationnelles des protéines de l'horloge. D'après (Chung, Son et al. 2011).

surrénalien par les NSC, et de la régulation circadienne des enzymes de la stéroïdogénèse par l'horloge moléculaire dans le cortex surrénalien (Chung, Son et al. 2011). Il a été ainsi décrit un effet direct de la lumière sur la sécrétion des glucocorticoïdes (Schibler and Brown

Figure 5. Exemple de rythme circadien du cortisol sérique chez l'Homme.

Exemple de rythme circadien normal du cortisol sérique (en $\mu\text{g/ml}$). On note le pic du cortisol en début de matinée, et des taux décroissants pendant la journée, avec des valeurs très basses pendant la nuit. Redessiné d'après (Kusanagi, Hida et al. 2008).



2005). La sécrétion rythmique du cortisol peut être modifiée par plusieurs facteurs exogènes regroupés sous le vocable de « stress » (McEwen 1998; Turner-Cobb, Sephton et al. 2000; Abercrombie, Giese-Davis et al. 2004; Giese-Davis, Wilhelm et al. 2006; Spiegel, Giese-Davis et al. 2006; Palesh, Zeitzer et al. 2008; Sephton, Dhabhar et al. 2009). Le rythme de sécrétion du cortisol chez l'Homme ou de la corticostérone chez la Souris ou le Rat synchronise directement les horloges circadiennes cellulaires des tissus périphériques, tant *in vivo* que *in vitro* (Balsalobre, Brown et al. 2000; Le Minh, Damiola et al. 2001; Stratmann and Schibler 2006; Kowalska and Brown 2007; Dibner, Schibler et al. 2010). Par ailleurs, les agents glucocorticoïdes synthétiques sont très largement utilisés en pratique clinique pour leurs nombreux effets thérapeutiques (Buttgereit, Doering et al. 2008; Hesketh 2008).

II.3.5. Autres rythmes

La pertinence des rythmes circadiens précédemment décrits pour l'évaluation du système circadien tient au fait qu'ils en reflètent la coordination centrale mais aussi qu'ils synchronisent effectivement les horloges des tissus de l'organisme.

Cependant, plusieurs autres rythmes circadiens existent chez l'Homme. Tout récemment ont été décrits les rythmes d'expression des gènes de l'horloge dans différents tissus humains, y compris chez les patients cancéreux (Levi, Focan et al. 2007; Okyar and Lévi 2008; Wood, Yang et al. 2009). Leur profil sont très similaires à ceux de leurs homologues murins, mais avec un décalage de phase d'environ 12 heures entre la Souris et l'Homme (Bjarnason, Jordan et al. 1999;

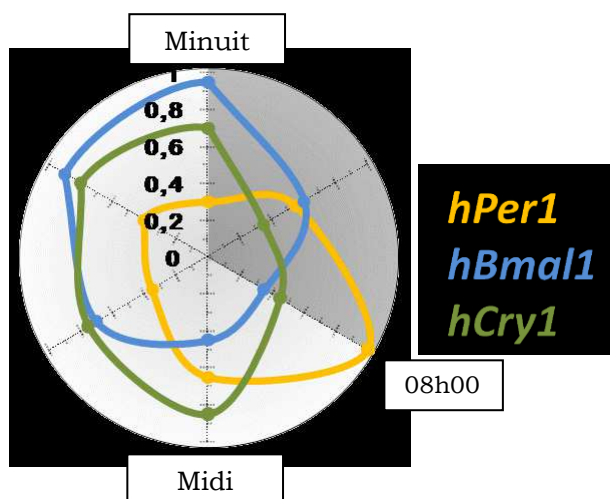


Figure 7. Exemple de rythme circadien de l'expression de trois gènes de l'horloge circadienne dans la muqueuse buccale humaine.

Exemple de rythme circadien de l'expression de l'ARNm de trois gènes de l'horloge : *hPer1* (orange), *hBmal1* (bleu) et *hCry1* (vert) dans la muqueuse buccale humaine (n=8 sujets sains males). Une biopsie a été prélevée toutes les quatre heures. Les valeurs sont normalisées à la valeur maximale. On note les trois acrophases décalées : à 08h30 pour *hPer1*, à 17h00 pour *hCry1* et à 21h30 pour *hBmal1*. Redessiné d'après (Bjarnason, Jordan et al. 2001).

Bjarnason,
Jordan et al.
2001;
Bjarnason
and Jordan
2002;
Kusanagi,
Hida et al.
2008). Ainsi,
par exemple,
la
transcription
en ARNm est
maximale en
fin de phase

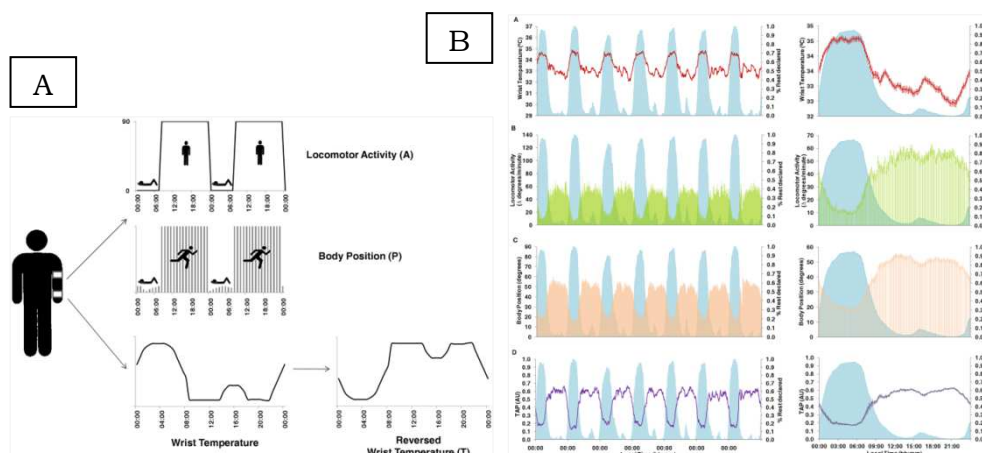


Figure 8. Exemple de paramètre complexe de rythmes circadiens chez l'Homme: TAP.

Panneau A: Localisation des senseurs utilisés pour mesurer les paramètres formant le TAP (c'est à dire: Température, Activité et Position), et représentation simplifiée des variables enregistrées. Panneau B: Enregistrement d'une semaine (n=49 sujets sains), avec toutes les variables mesurées et le paramètre TAP résultant, par rapport à la phase de sommeil (en bleu clair). D'après (Ortiz-Tudela, Martinez-Nicolas et al. 2010).

de repos pour les gènes *Per* et en fin de phase d'activité pour *Bmal1* dans les tissus périphériques de la Souris et de l'Homme (Figure 13). La mesure de ces rythmes moléculaires nécessite six à huit prélèvements quotidiens, réservant leurs déterminations à des études translationnelles d'effectif limité.

Enfin, pour améliorer la précision dans l'estimation de la phase de chaque individu, il a été proposé d'enregistrer conjointement plusieurs rythmes, pour en dériver un paramètre complexe intégrant les informations dérivées de chaque rythme étudié. Un exemple de ce type d'approche, potentiellement intéressant mais multipliant les dispositifs de mesure, risquant d'entraver l'application dans le champ de l'oncologie, a été rapporté pour l'activité, la position corporelle et la température cutanée (Figure 14) (Ortiz-Tudela, Martinez-Nicolas et al. 2010). Ce type d'investigation fait l'objet d'une coopération en cours chez les patients cancéreux ambulatoires entre notre Unité et l'Université de Murcia, en España, qui a développé cette approche.

II.4. Relations entre le système circadien et le cancer

II.4.1. Effet du système circadien sur la tumeur

II.4.1.1. Evidence expérimentale

Le rôle de la disruption circadienne dans la cancérogenèse et la progression tumorale s'appuie sur un grand nombre de données mécanistiques, expérimentales, épidémiologiques et cliniques. Cet ensemble a conduit l'Agence Internationale de Recherche sur le Cancer de l'Organisation Mondiale de la Santé à considérer le travail posté, qui provoque une disruption circadienne, comme facteur de risque de cancer de classe IIA, c'est-à-dire proche de l'évidence complète (Straif, Baan et al. 2007; Stevens, Hansen et al. 2011). A la suite de cette expertise internationale, le Danemark est devenu le premier pays à considérer le cancer du sein comme maladie professionnelle secondaire au travail posté, en l'absence d'autre facteur connu de risque et à la condition d'au moins un poste nocturne chaque semaine pendant 20 ans (Wise 2009). En conséquence, plusieurs demandes légales de remboursement ont été acceptées par ce Membre de la Communauté Européenne (Wise 2009). En France, la Haute Autorité de Santé a tout récemment commissionné la rédaction d'un ensemble de Recommandations de Bonne Pratique sur le thème « Surveillance médico-professionnelle des travailleurs postés et en horaires atypiques » (2011). Ces actions témoignent de la prise de conscience au niveau des Institutions Officielles de l'importance des rythmes biologiques pour la physiopathologie humaine, en particulier celle qui concerne le cancer.

L'abondante littérature scientifique montrant l'effet néfaste de la disruption circadienne pour différentes pathologies humaines a été résumée de façon exhaustive par l'OMS pour ce qui concerne le cancer (Straif, Baan et al. 2007; Stevens, Hansen et al. 2011), et par plusieurs auteurs pour les troubles du comportement, les troubles cardio-vasculaires, et le syndrome métabolique (Penev, Kolker et al. 1998; Rajaratnam and Arendt 2001; Turek, Dugovic et al. 2001; Barger, Lockley et al. 2009; Scheer, Hilton et al. 2009; Arble, Ramsey et al. 2010; Reddy and O'Neill 2010; Etain, Milhiet et al. 2011; Harvey 2011; Huang, Ramsey et al. 2011). Pour le cancer, les aspects mécanistiques ont été aussi décrits par plusieurs équipes dans le monde (Chen-Goodspeed and Lee 2007; Gery and Koeffler 2007; Levi, Filipinski et al. 2007). Je montre ici deux exemples pertinents pour ma thèse.

Chez la Souris, la croissance tumorale est accélérée par l'induction d'une disruption circadienne provoquée par la destruction physique du pacemaker central ou sa suppression fonctionnelle (Filipski, King et al. 2002; Filipski, Delaunay et al. 2004; Filipski, Innominato et al. 2005; Filipski, Li et al. 2006; Filipski and Levi 2009) (Figure 15). Au cours d'un stage de recherche réalisé avant le début de ma thèse, j'ai contribué à l'identification des mécanismes moléculaires impliqués dans l'accélération de la progression tumorale chez des souris exposées à un décalage horaire chronique, et montré qu'une alimentation programmée contrebalançait cet effet. Ces résultats ont souligné le principe que le renforcement du système circadien pouvait ralentir l'évolutivité du cancer (Filipski, Innominato et al. 2005). Notre Unité a montré que la dérégulation de *c-Myc* et la répression de *P53* étaient une conséquence de la disruption de l'horloge circadienne qui rendait compte de l'accélération de la progression cancéreuse (Filipski, Innominato et al. 2005). Ces mêmes résultats ont été retrouvés chez des souris présentant une mutation du gène de l'horloge *Per2* par une équipe américaine (Fu, Pelicano et al. 2002).

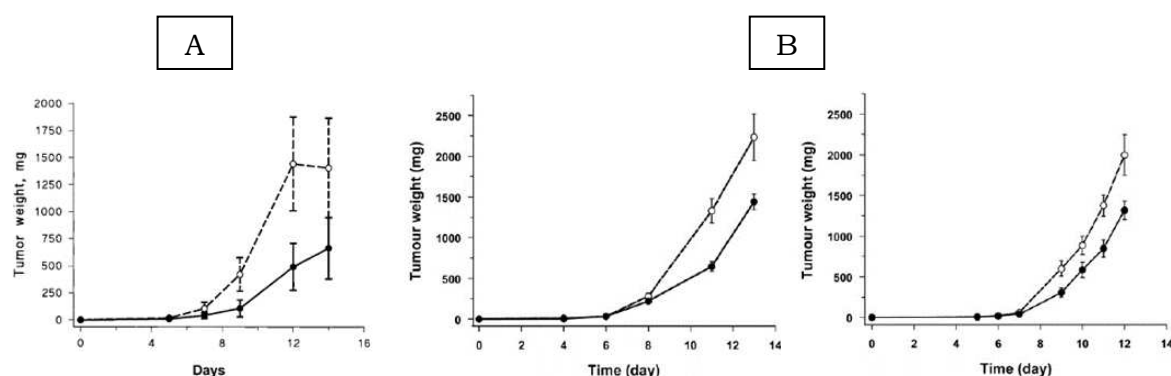


Figure 9. Courbes de croissance tumorale chez la Souris en présence de disruption du système circadien

Courbes de croissance tumorale (tumeur GOS: Glasgow Osteosarcoma, implanté) chez la Souris en présence de disruption du système circadien (cercles blancs vides), soit par destruction anatomique stéréotaxique des Noyaux Suprachiasmatiques (NSC) (panneau A), soit par altération fonctionnelle obtenue par une exposition prolongée à un décalage horaire chronique (seulement 4 heures d'obscurité chaque 48 heures) (panneaux B, deux expérimentations indépendantes). Les cercles noirs montrent les souris de contrôle (craniotomie sans ablation des NSC ou exposition à 12 heures de lumière et 12 heures d'obscurité). On note une accélération significative de la croissance tumorale induite par la disruption du système circadien (analyse de variance: respectivement $p=0,004$; $p<0,001$ et $p=0,002$). D'après (Filipski, King et al. 2002; Filipski, Delaunay et al. 2004).

II.4.1.2. Données cliniques

Les résultats expérimentaux précédemment décrits se traduisent en clinique par la démonstration d'une moindre survie des patients atteints de cancer avancé qui

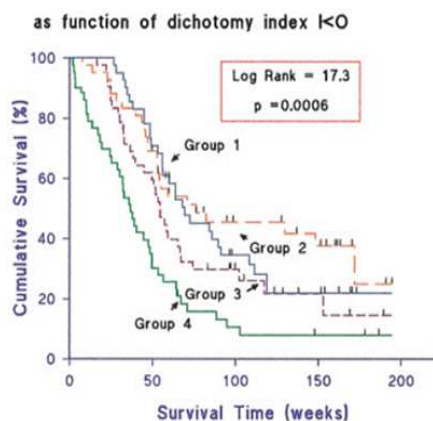


Figure 11. Courbes de Kaplan-Meier de survie globale en fonction du rythme circadien d'activité-repos chez des patients atteints de cancer colorectal métastatique.

Estimation de la durée de survie globale de 192 patients atteints de cancer du côlon ou rectum métastatique selon le rythme circadien d'activité et repos, estimé par actimétrie du poignet. La perturbation du rythme circadien d'activité et repos est mesuré par l'index de dichotomie I<O et les quatre courbes représentent la survie des patients selon leur quartile de I<O: 1^{er} (le plus bas): vert; 2^{ème} : violet; 3^{ème} : rouge; 4^{ème} (le plus haut): bleu. Valeur du p du Log Rank: 0,0006. D'après (Mormont, Waterhouse et al. 2000; Mormont and Levi 2003).

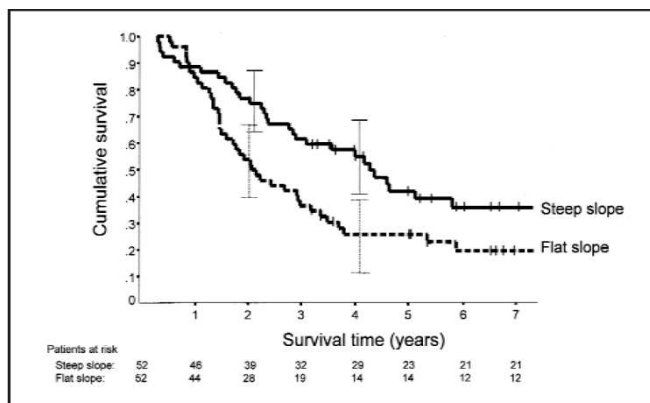


Figure 10. Courbes de Kaplan-Meier de survie globale en fonction du rythme circadien du cortisol salivaire chez des femmes atteintes de cancer mammaire métastatique.

Estimation de la durée de survie globales de 104 patientes atteintes de cancer du sein métastatique selon le rythme circadien du cortisol salivaire: ligne continue (n=77): rythme normal conservé; ligne discontinue (n=77): rythme altéré. Valeur du p du Log Rank: 0,016. D'après (Sephton, Sapolsky et al. 2000).

présentent une disruption circadienne avant chimiothérapie. La valeur pronostique négative de la disruption du rythme circadien du cortisol salivaire dans le cancer du sein et celle du rythme circadien d'activité locomotrice dans le cancer colorectal sont indépendantes des facteurs pronostiques connus (Mormont, Waterhouse et al. 2000; Sephton, Sapolsky et al. 2000). De plus, il faut noter que l'effet délétère de la disruption circadienne sur la survie globale ne se limite pas aux semaines ou mois qui suivent la mesure, mais persiste plusieurs années (figures 16 et 17). Ceci confirme l'importance et l'indépendance de l'information sur le rythme circadien pour le pronostic du patient.

II.4.2. Effet du cancer sur la physiologie circadienne

II.4.2.1. Disruption circadienne chez les patients cancéreux

L'incidence précise d'altération fonctionnelle ou constitutive du système circadien des patients atteints de cancer reste à préciser. L'existence d'une disruption des rythmes circadiens des patients cancéreux a été rapportée par différentes équipes en situations cliniques variées (Focan, Focan-Henrard et al. 1986; Smaaland, Abrahamsen et al. 1992; Smaaland, Lote et al. 1993; Touitou, Levi et al. 1995;

Touitou, Bogdan et al. 1996; Wood and Hrushesky 1996; Mormont and Levi 1997; Berger 1998; Hrushesky, Lannin et al. 1998; Mormont, Hecquet et al. 1998; Berger and Farr 1999; Mazzocchi, Balzanelli et al. 1999; Mazzocchi, Giuliani et al. 1999; Berger and Higginbotham 2000; Mormont, Waterhouse et al. 2000; Sephton, Sapolsky et al. 2000; Turner-Cobb, Sephton et al. 2000; Ancoli-Israel, Moore et al. 2001; Hrushesky 2001; Berger, VonEssen et al. 2002; Levi 2002; Mormont, Bogdan et al. 2002; Mormont, Langouet et al. 2002; Mormont and Waterhouse 2002; Smaaland, Sothorn et al. 2002; Berger, VonEssen et al. 2003; Lis, Grutsch et al. 2003; Mazzocchi, Carughi et al. 2003; Mazzocchi, Grilli et al. 2003; Mormont and Levi 2003; Sephton and Spiegel 2003; Abercrombie, Giese-Davis et al. 2004; Lee, Cho et al. 2004; Levin, Daehler et al. 2005; Mazzocchi, Carughi et al. 2005; Rich, Innominato et al. 2005; Ancoli-Israel, Liu et al. 2006; Giese-Davis, DiMiceli et al. 2006; Giese-Davis, Wilhelm et al. 2006; Spiegel, Giese-Davis et al. 2006; Berger, Farr et al. 2007; Fouladiun, Korner et al. 2007; Iurisci, Rich et al. 2007; Levi, Focan et al. 2007; Lévi and Schibler 2007; Berger, Wielgus et al. 2008; Lutgendorf, Weinrib et al. 2008; Miller, Ancoli-Israel et al. 2008; Palesh, Zeitzer et al. 2008; Spiegel 2008; Berger 2009; Berger, Kuhn et al. 2009; Berger, Kuhn et al. 2009; Berger, Wielgus et al. 2009; Du-Quiton, Wood et al. 2009; Guess, Burch et al. 2009; Liu, Fiorentino et al. 2009; Sephton, Dhabhar et al. 2009; Singletary, Wood et al. 2009; Berger, Grem et al. 2010; Eismann, Lush et al. 2010; Mazzocchi, Vendemiale et al. 2010; Palesh, Roscoe et al. 2010; Weinrib, Sephton et al. 2010; Mazzocchi 2011; Mazzocchi, Fontana et al. 2011; Mazzocchi, Paziienza et al. 2011; Mazzocchi, Sothorn et al. 2011; Mazzocchi, Sothorn et al. 2011; Mazzocchi, Tarquini et al. 2011; Payne 2011). L'hétérogénéité des études cliniques en termes de stade et type de maladie néoplasique, mais surtout de rythmes biomarqueurs évalués, de leurs outils de mesure et du choix des critères d'anormalité, limite en effet l'estimation précise de la fréquence d'une disruption circadienne chez les patients cancéreux. Ce manque d'information appelle la mise en place d'études systématiques et collaboratives dans ce domaine. Pour l'évaluation de l'incidence des troubles du sommeil, pour partie liés à l'altération du système circadien, ce type d'effort commence à favoriser la prise de conscience médicale de l'ampleur du problème et à promouvoir la recherche d'intervention thérapeutique (Savard and Morin 2001; Parker, Bliwise et al. 2008; Spiegel 2008; Berger, Kuhn et al. 2009; Savard, Villa et al. 2009; Palesh, Roscoe et al. 2010; Savard, Ivers et al. 2011).

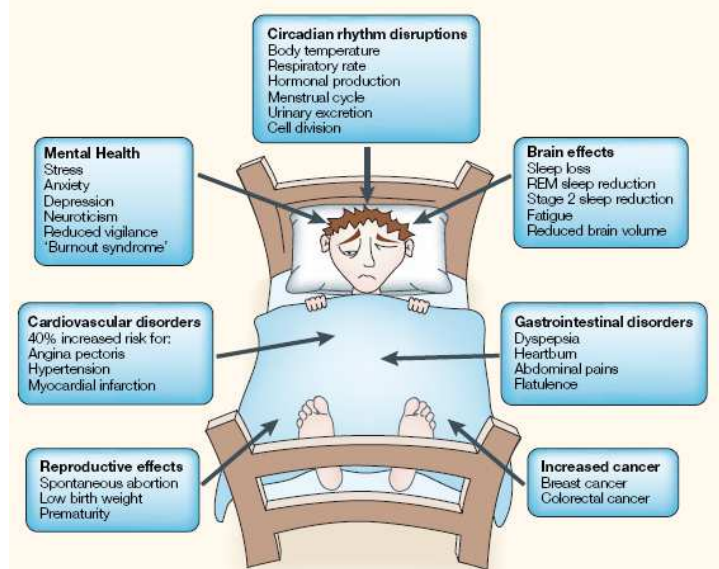
II.4.2.2. Symptômes corrélés à la disruption circadienne

Si les données précises sur l'incidence de la disruption circadienne en cas de cancer font encore défaut, le rôle du système circadien dans le bien-être de l'individu est mieux connu, surtout grâce aux études réalisées chez les sujets présentant une désynchronisation circadienne secondaire au travail posté ou à un décalage horaire après vol transmériidien long courrier. En effet, la désynchronisation entre le pacemaker interne et l'environnement cyclique externe est responsable d'une perte de qualité et de quantité de sommeil, et de l'apparition d'autres symptômes qui concernent tous les systèmes contrôlés par le système circadien (Figure 18). Ces symptômes sont particulièrement retrouvés chez les sujets exposés à un travail posté ou à un décalage horaire excédant 4 h (Waterhouse, Reilly et al. 1997; Drake, Roehrs et al. 2004; Foster and Wulff 2005; Waterhouse, Reilly et al. 2007).

L'hypothèse que l'altération de la fonction circadienne soit un des mécanismes de certains symptômes chez les patients atteints de cancer et/ou en cours de chimiothérapie est évaluée dans mon travail de thèse.

Figure 12. Symptômes associés à la disruption circadienne chez des sujets sans cancer.

Illustration des nombreux problèmes physiologiques et mentaux liés à la disruption circadienne induite par le travail posté ou le décalage horaire. D'après (Foster and Wulff 2005).



II.5. Principes de chronopharmacologie des cancers

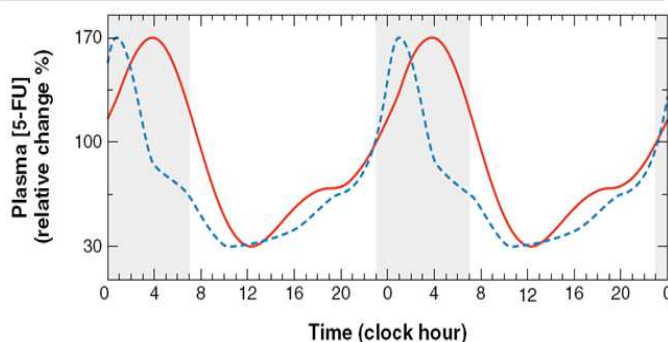
II.5.1. Principes généraux de chronopharmacologie

II.5.1.1. Chronopharmacocinétique

Les études de pharmacocinétique permettent de décrire et modéliser les variations temporelles des concentrations du médicament et de ses métabolites dans le sang et les tissus. Le profil pharmacocinétique d'un médicament est le résultat de plusieurs processus biologiques tels que l'absorption, la distribution, la

Figure 13. Exemple de chronopharmacocinétique du 5-fluorouracile chez des patients atteints de cancer.

Évolution dans le temps des concentrations plasmatiques de 5-fluorouracile (5-FU) chez 11 patients cancéreux recevant une perfusion à débit constant par voie intraveineuse pendant 5 jours. Les échantillons de plasma ont été obtenus le premier, le troisième et le cinquième jour d'une perfusion constante de 5-FU comme agent unique (étude #1) ou de l'association de 5-FU, d'acide folinique et d'oxaliplatine (étude #2). Pour des raisons graphiques, les profils de 24 h ont été dupliqués. On note le profil chronopharmacocinétique du 5-FU avec pic plasmatique pendant la nuit, malgré une administration à débit constant. D'après (Lévi and Schibler 2007).



biotransformation et l'excrétion.

La plupart des fonctions biologiques qui déterminent la pharmacocinétique des médicaments présentent des variations circadiennes (Lévi and Schibler 2007), responsables de modifications du profil pharmacocinétique en fonction du moment d'administration du médicament. Une telle chronopharmacocinétique a été démontrée chez l'Homme pour plus de 57 médicaments, de toute classe pharmacologique, y compris pour au moins 7 agents cytotoxiques anticancéreux (Koukkari and Sothorn 2006;

Lévi and Schibler 2007).

Ses mécanismes font intervenir le contrôle circadien qui affecte notamment la sécrétion biliaire, la vidange gastrique, la motilité intestinale, le débit sanguin dans les organes digestifs, la liaison aux protéines plasmatiques, la fluidité membranaire et les processus de fixation érythrocytaires, la biotransformation microsomale par les cytochromes et par d'autres activités enzymatiques, les réactions de conjugaison, les transporteurs transmembranaires, et la filtration glomérulaire et la sécrétion tubulaire rénale (Koukkari and Sothorn 2006; Lévi and Schibler 2007).

Bien que peu nombreuses, les études de chronopharmacocinétique des cytostatiques ont mis en évidence des variations circadiennes intra-individuelles du même ordre de grandeur que les différences interindividuelles. L'exemple le mieux étudié concerne le 5-fluorouracile. Cet antimétabolite utilisé dans nombreux schémas thérapeutiques de différents cancers (Longley, Harkin et al. 2003) présente un profil chronopharmacocinétique plasmatique avec variations circadiennes démontrées dans plusieurs études chez l'Homme (Lévi and Schibler 2007) (Figure 19). Dans une autre étude clinique circadienne, on observe un triplement de la concentration plasmatique moyenne du 5-FU, administré en perfusion constante à la dose de 300 mg/m², au cours des 24 h (Harris, Song et al. 1989). Une autre étude indique la nécessité de tripler la dose de 5-FU perfusée en 8 h (de 9h00 à 17h00) pour atteindre une concentration plasmatique cible de [5-FU] entre 2,5 et 3,0 mg/l selon les patients (Gamelin, Delva et al. 2008).

II.5.1.2. Chronopharmacodynamie

La pharmacodynamie étudie les effets d'un médicament sur l'organisme. Les variations temporelles circadiennes des interactions entre la forme active du médicament (avec une concentration donnée au site concerné) et ses cibles moléculaires déterminent la magnitude et la durée d'action observée au niveau moléculaire, cellulaire et de l'organisme. L'action pharmacologique d'un médicament en fonction du moment d'administration ne dépend pas seulement de son profil chronopharmacocinétique, mais aussi des variations circadiennes d'autres paramètres, tels que la densité des récepteurs ou des activités enzymatiques cibles et des mécanismes de détoxification, de réparation ou d'excrétion (Lévi and Schibler 2007).

Les variations circadiennes de la pharmacocinétique des médicaments et celles de leur pharmacodynamie rendent compte des notions de chronoefficacité et de chronotolérance.

II.5.2. Chronopharmacologie expérimentale des agents anticancéreux

II.5.2.1. Chronotolérance

Il a été observé que le système circadien modifiait de façon importante la toxicité de 40 médicaments anticancéreux, y compris les cytostatiques, les cytokines et les agents biologiques ciblés, chez la souris ou le rat (Levi 1999; Lévi 2001; Levi 2002;

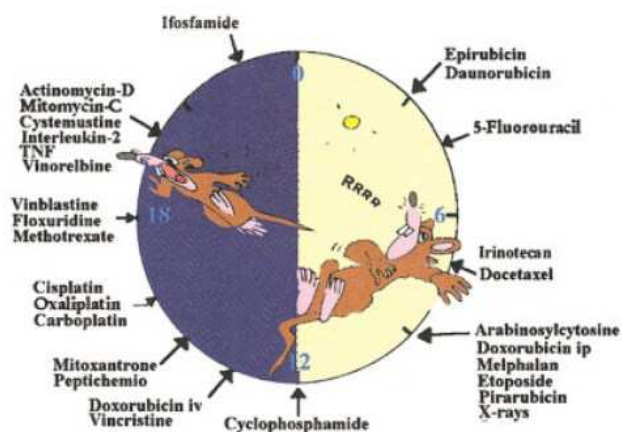


Figure 14. Chronotolérance des agents anticancéreux chez la Souris ou le Rat

Rythmes circadiens de la tolérance de plus de 30 médicaments anticancéreux chez des souris ou des rats de laboratoire. L'heure d'administration le moins toxique est indiquée pour chaque agent cytostatique ou immunologique en fonction du cycle circadien d'activité/repos. Pour tous ces médicaments, les taux de survie signalés varient de plus de 50% selon l'heure d'administration d'une dose potentiellement létale. Une telle différence importante est observée indépendamment de la voie d'injection (intraveineuse ou intrapéritonéale) ou du nombre d'injections (unique ou répétée). D'après (Mormont and Levi 2003).

Mormont and Levi 2003; Levi 2006) (Figure 20). Une dose potentiellement létale de n'importe lequel de ces agents entraîne un changement de l'incidence de décès toxique et/ou de la perte pondérale maximale de 2 à 10 fois en fonction de l'heure circadien d'administration (Levi 1999; Lévi 2001; Levi 2002; Mormont and Levi 2003; Levi 2006; Lévi and Schibler 2007).

Ces rythmes de toxicité de grande amplitude se produisent indépendamment de la voie d'administration: orale, intraveineuse, intrapéritonéale ou intra-artérielle, ou du nombre d'administrations quotidiennes ou

hebdomadaires (Levi 1999; Lévi 2001; Levi 2002; Mormont and Levi 2003; Levi 2006; Lévi and Schibler 2007).

La méthodologie utilisée pour démontrer les changements de tolérance au cours des 24 heures implique la synchronisation des animaux nocturnes par l'alternance de 12 heures de lumière et de 12 heures d'obscurité (LO12:12). Une dose égale du médicament est administrée aux différents groupes de rats ou de souris, avec chaque groupe correspondant à un stade différent du rythme circadien, référé à un donneur de temps externe ou Zeitgeber (ZT). Habituellement, au moins six stades circadiens à 4 heures d'intervalle, sont testés. Le temps est généralement exprimé en heures ZT ou en heures Après le Début de la Lumière (hADL). Les horaires circadiens optimaux s'échelonnent tout au long des 24 heures pour les différentes molécules testées (Figure 20). Ils ne peuvent pas être prévus à ce jour par la connaissance de la classe pharmacologique ou des principaux organes cibles de la toxicité. Les rythmes circadiens de tolérance aux anticancéreux persistent chez les rongeurs maintenus en obscurité ou en lumière constantes; ceci démontre leur

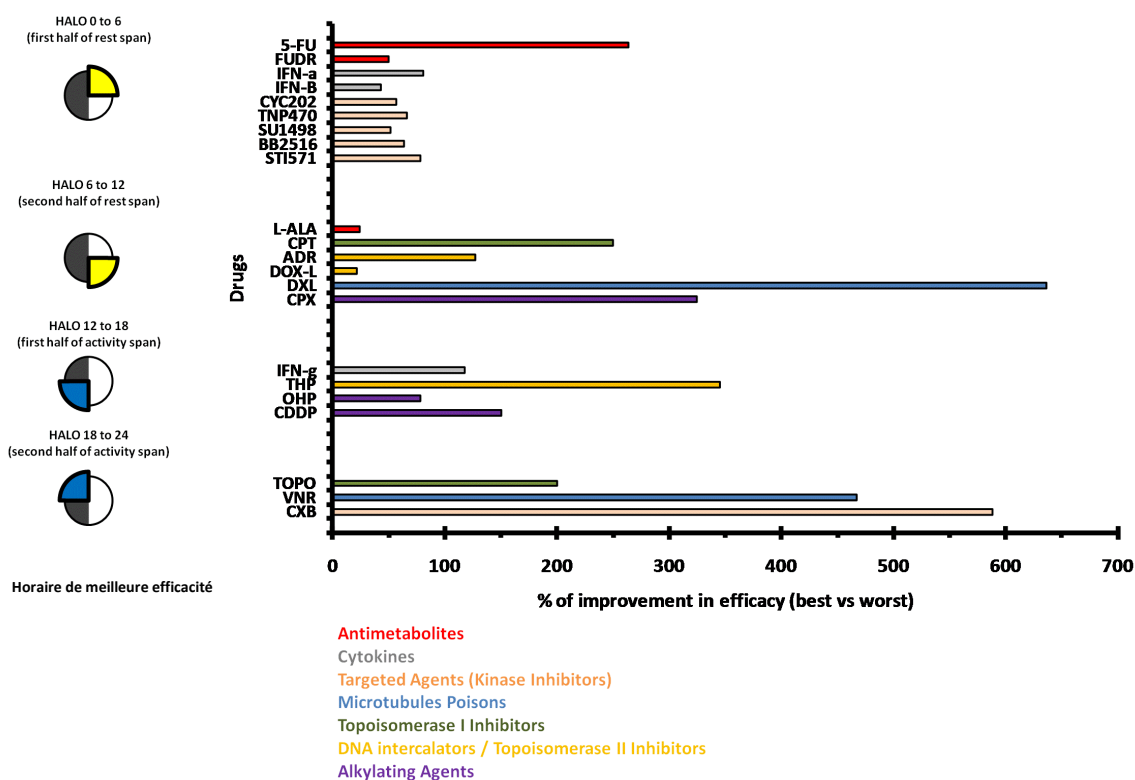
endogénicité (Levi 1999; Lévi 2001; Levi 2002; Mormont and Levi 2003; Levi 2006; Lévi and Schibler 2007).

II.5.2.2. Chronoefficacité

Il a aussi été observé que le système circadien contrôlait de façon importante l'efficacité antitumorale de 28 médicaments anticancéreux, y compris les cytostatiques, les agents anti-angiogéniques, et les inhibiteurs du cycle cellulaire ou de la cyclooxygénase-2 (Cox2) chez les rongeurs porteurs de divers types de tumeurs malignes (Levi 1999; Lévi 2001; Granda and Levi 2002; Levi 2002;

Figure 15. Chronoefficacité des agents anticancéreux chez la Souris ou le Rat.

Pourcentage de l'amélioration de l'activité antitumorale (mesurée comme durée de survie, taux de rémission ou ralentissement de la vitesse de croissance) de différents agents anticancéreux administrés aux horaires meilleurs par rapport aux pires. Ces différences représentent la démonstration d'un profil de chronoefficacité du médicament testé. Les différentes classes de médicaments anticancéreux sont représentées par la même couleur. Les horaires de meilleure activité du médicament ont été groupés en quatre périodes des six heures sur les 24 heures par rapport au début de la lumière (HALO: Hours After Light Onset).



Mormont and Levi 2003; Levi 2006; Lévi and Schibler 2007) (Figure 21). La démonstration de la chronoefficacité est basée sur l'administration d'un agent anticancéreux seul ou combiné à un ou plusieurs autre(s) anticancéreux pendant plusieurs jours ou semaines à des stades circadiens définis (Levi 1999; Lévi 2001; Granda and Levi 2002; Levi 2002; Mormont and Levi 2003; Levi 2006). Une chimiothérapie, avec un ou plusieurs médicaments, à doses et horaires circadiens appropriés, diminue d'au moins la moitié la vitesse de croissance tumorale et/ou

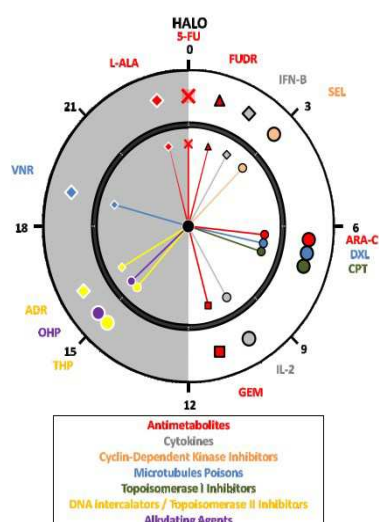


Figure 16. Correspondance entre horaire d'administration le moins toxique et le plus efficace pour 14 agents anticancéreux chez les rongeurs porteurs de tumeurs.

Représentation des horaires d'administration des médicaments anticancéreux correspondant à une moindre toxicité (cercle interne) et ceux correspondant à une efficacité supérieure (cercle externe). Les classes des agents anticancéreux sont identifiées par la même couleur, et chaque médicament par le même symbole. On note la parfaite correspondance entre horaire de meilleure tolérance et horaire de meilleure efficacité pour tous les médicaments testés, au cours des différentes expérimentations.

augmente significativement la durée de vie des souris porteuses de tumeurs (Levi 1999; Lévi 2001; Granda and Levi 2002; Levi 2002; Mormont and Levi 2003; Levi 2006). Le système circadien modifie également largement l'efficacité des agents anticancéreux utilisés contre les cellules tumorales humaines du sein (MCF-7, ZR-75-30, et MDA-MB-468) ou du côlon (HCT116) transplantées chez des souris nues athymiques (Lévi and Schibler 2007).

Comme pour la chronotolérance, les meilleurs horaires de chronoefficacité des médicaments anticancéreux s'échelonnent tout au long de la période des 24 heures, indépendamment de leur classe pharmacologique (Figures 21 et 22) (Levi 1999; Lévi 2001; Levi 2002; Mormont and Levi 2003; Levi 2006).

II.5.2.3. Corrélation entre chronotolérance et chronoefficacité

Étonnamment, le profil circadien de la chronoefficacité d'un anticancéreux coïncide généralement avec celui de sa chronotolérance (Figure 22). Cela est vrai pour les cytostatiques, les interférons, les agents anti-angiogéniques, et

les inhibiteurs du cycle cellulaire, ainsi que pour les associations de médicaments, telles que irinotécan-oxaliplatine, gemcitabine-cisplatine et docétaxel-doxorubicine, trois schémas largement utilisés en clinique (Granda, Filipinski et al. 2001; Granda, D'Attino et al. 2002; Li, Tanaka et al. 2005; Perez 2005; Sobrero 2009; Martin, Segui et al. 2010; Valle, Wasan et al. 2010; Conroy, Desseigne et al. 2011).

La chronothérapie expérimentale, démontrant des profils de chronotolérance et de chronoefficacité pour les médicaments utilisés en clinique, ou même pendant leur développement pré-clinique, considère donc la nécessité d'administrer les médicaments anticancéreux à un stade circadien précis pour améliorer l'index thérapeutique.

II.5.3. Chronothérapeutique des cancers humains

Plusieurs données cliniques sont maintenant disponibles qui montrent le rôle de l'horaire d'administration d'une chimiothérapie par 5-Fluorouracile ou floxuridine seuls, ou combinant cisplatine et doxorubicine ou theprubicine, 5-fluorouracile-acide folinique avec ou sans oxaliplatine ou carboplatine, la sur ses toxicités et/ou son intensité de dose (Tableau IV) (Bjarnason and Hrushesky 1992; Hrushesky and Bjarnason 1993; Hrushesky 1995).

Cette stratégie d'optimisation de l'horaire d'administration peut améliorer aussi la réponse tumorale et/ou la survie globale chez les patients atteints de cancer (Lévi 2001; Mormont and Levi 2003). La démonstration clinique de la prise en charge des patients cancéreux a été menée principalement dans le cadre du traitement du cancer colorectal métastatique par l'association d'oxaliplatine, de 5-fluorouracile et d'acide folinique (Tableau V).

Tableau IV. Liste des essais cliniques en oncologie avec horaire d'administration établi.
Modifiée d'après (Hrushesky and Bjarnason 1993).

Type d'essai	Médicaments	# pts.	Type de cancer	Schéma/Horaires testés	Résultats
Phase-I/II/III.	FUDR.	36	Avancé	Perfusion continue vs circadienne (14 jours)	Perfusion circadienne moins toxique.
Phase-II.	FUDR.	68	Rein	Perfusion circadienne (14 jours); pic à 15-21h.	RC + RP = 20%.
Phase-II.	FUDR.	42	Rein	Perfusion circadienne (14 jours); pic à 15-21h.	RC + RP = 14%.
Phase-II.	FUDR.	42	Rein.	Perfusion circadienne (14 jours); pic à 15-21h.	RP = 10% (+10% sur les métastases).
Phase-II	FUDR	13	Rein	Perfusion circadienne (14 jours); pic à 15-21h.	RC + RP = 62%
Phase-III.	FUDR i.h.	50	Colorectal	Perfusion continue vs circadienne (14 jours).	Même taux de réponse: 32-35%
Phase-III.	5-FU i.v. FUDR i.h.	38	Colorectal	Perfusion continue vs circadienne (5 jours).	Même taux de réponse: 50-60%. Survie médiane supérieure dans le bras chrono: 40+ vs 19 mois.
Phase-I.	5-FU.	35	Colorectal	Perfusion circadienne (5 jours); pic à 04h.	Dose-intensité supérieure de 75% par rapports aux données historiques.
Phase-I.	5-FU. LV.	14	Avancé	Perfusion circadienne (14 jours); pic à 04h vs 22h.	Pic à 22h moins toxique.
Phase-II.	5-FU. LV. oxaliplatine.	93	Colorectal	Perfusion circadienne (5 jours).	Taux de réponse: 58%.
Phase-II.	5-FU. LV. oxaliplatine.	37	Colorectal	Perfusion circadienne (5 jours).	Taux de réponse: 43%.
Phase-II	5-FU. LV. oxaliplatine.	48	Colorectal	Perfusion circadienne (4 jours).	Taux de réponse: 38%
Phase-III.	Doxorubicine. Cisplatine.	23	Ovaire, Vessie	A. Dox. 06h. Cispl. 18h. vs B. Dox. 18h. Cispl. 06h.	Schéma A moins toxique.
Phase-III.	Doxorubicine. Cisplatine.	37	Ovaire	A. Dox. 06h. Cispl. 18h. vs B. Dox. 18h. Cispl. 06h.	Schéma A moins toxique. Meilleure survie avec schéma A.
Phase-II.	Doxorubicine. Cisplatine.	25	Endomètre	Dox. 06h. Cispl. 18h.	RC + RP: 16% + 36%.
Phase-II Randomisé	THP-Doxoru Cisplatine	31	Ovaire	A. THP 06h. Cispl. 16-20h. B. THP 18h. Cispl. 16-20h.	Taux de réponse: A:73% vs B:57% Schéma A moins toxique.
Phase-I.	Oxaliplatine.	23	Avancé	Perfusion continue vs circadienne (5 jours).	Perfusion circadienne moins toxique.
Phase-I (crossover).	Carboplatine.	7	Ovaire	Bolus 400 mg/m ² à 06h vs 18h.	Moindre thrombocytopénie à 18h.
Phase-II Randomisé	Etoposide Cisplatine	34	Métastatique	VP-16 à 07h ou 15h. Cisplatine à 18h.	Moindre toxicité médullaire à 07h.
Phase-III.	Etoposide. Cisplatine.	124	Poumon	Cisplatine à 18h. VP-16 à 06h ou 18h.	Moindre toxicité médullaire à 07h. Même taux de réponse.
Phase-I.	α-Interferon.	10	Melanoma. Rein	Perfusion circadienne (21 jours); pic à 18-22h.	Dose-intensité supérieure par rapports aux données historiques.
Revue Rétrospective	6-MP. MTX.	118	LAL	Traitement le matin vs le soir.	Avantage en survie pour le groupe du soir.

Tableau V. Chronologie du développement clinique des protocoles chronomodulés associant 5-Fluorouracile, Leucovorine et Oxaliplatine.

Phase d'essai	# pts.	Médicament(s)	Schéma/Horaire	Résultats	Ref.
I- Randomisé	23	Oxaliplatine	Perfusion de 5 jours circadienne (pic à 16h) vs continue	Meilleure tolérance du schéma chrono	JNCI 1990
I	34	5-FU/LV	Perfusion circadienne de 5 jours, pic à 04h	Escalade de dose possible, avec bonne tolérance	EJC 1997
II	100	5-FU/LV	Perfusion circadienne de 4 jours, pic à 04h	TR: 41% TPM: 7 mois SG: 17 mois Chirurgie: 20% Bonne tolérance	JCO 2002
II	30	Oxaliplatine	Perfusion circadienne de 5 jours, pic à 16h	TR: 10% Bonne tolérance	EJC 1993
II	93	Oxaliplatine, 5-FU/LV	chronoFLO5	TR: 58% TPM: 10 mois SG: 15 mois Chirurgie: 13% Bonne tolérance	C 1992
II	50	Oxaliplatine, 5-FU/LV	chronoFLO4	TR: 48% TPM: 9.3 mois SG: 17.8 mois Chirurgie: 26% Bonne tolérance	JCO 1996
II	90	Oxaliplatine, 5-FU/LV	chronoFLO4	TR: 66% TPM: 8.4 mois SG: 18.5 mois Chirurgie: 34% Bonne tolérance	C 1999
I	114	Oxaliplatine, 5-FU/LV	chronoFLO4 décalé	Meilleure tolérance avec le schéma chronoFLO4 standard	ADDR 2007
III	200	5-FU/LV ± Oxaliplatine	Perfusion circadienne de 5 jours de 5-FU/LV (pic à 04h) ± 1-OHP le J1	Augmentation du TR et du TPM (mais pas de la SG) avec 1-OHP, bonne tolérance globale (G3-4 ≤ 43%)	JCO 2000
III	92	Oxaliplatine, 5-FU/LV	chronoFLO5 vs perfusion continue	Meilleure activité (TR, TPM, SG) et meilleure tolérance dans le bras chrono	JNCI 1994
III	186	Oxaliplatine, 5-FU/LV	chronoFLO5 vs perfusion continue	Meilleure activité (TR, TPM, SG) et meilleure tolérance dans le bras chrono	TL 1997

Les bases expérimentales qui ont conduit à concevoir le schéma d'administration chronomodulée de l'association de 5-Fluorouracile-Acide folinique (ou Leucovorin)-Oxaliplatine (chronoFLO) (Figure 23) proviennent des études de chronotoxicité de ces agents réalisées chez les souris mâles, de données translationnelles de chronopharmacocinétique humaine du 5-fluorouracile ou de l'oxaliplatine et leurs mécanismes, qui ont principalement concerné des patients de sexe masculin (Caussanel, Lévi et al. 1990; Lévi, Misset et al. 1992; Levi, Perpoint et al. 1993;

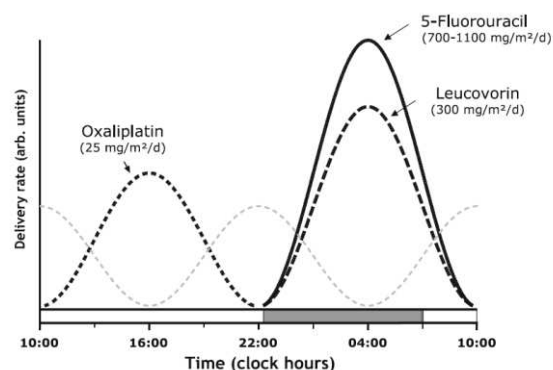


Figure 18. chronoFLO.

Profil de perfusion chronomodulée de 5-fluorouracile, d'acide folinique et d'oxaliplatine sur 24 h. Ce cycle est souvent répété automatiquement pendant 4 ou 5 jours consécutifs toutes les deux à trois semaines, en utilisant une pompe multicanaux programmable dans le temps. Ce schéma thérapeutique est utilisé pour traiter les patients atteints de cancer colorectal à leur maison, pendant leurs activités habituelles. D'après (Levi 2006).

Lévi, Zidani et al. 1994; Bertheault-Cvitkovic, Jami et al. 1996; Garufi, Levi et al. 1997; Lévi, Zidani et al. 1997; Lévi, Zidani et al. 1999; Giacchetti, Perpoint et al. 2000; Cure, Chevalier et al. 2002; Levi, Focan et al. 2007).

Le développement clinique de l'association chronoFLO, et la pertinence clinique du principe de chronothérapie, ont donc été conduits dans une large population de patients atteints de cancer colorectal métastatique selon la méthodologie standard des essais cliniques de Phase I, II - randomisé ou non-, et de Phase III randomisés, contrôlés.

Les différents essais, souvent internationaux, n'ont pas seulement permis d'identifier la dose et l'horaire optimaux d'administration des trois agents combinés, ainsi que la pertinence clinique de leur développement avec une importante activité antitumorale, mais ils ont aussi décrit et classifié la neurotoxicité périphérique sensitive liée à l'oxaliplatine, et démontré la faisabilité de l'administration de chimiothérapies complexes au domicile du patient. Les recherches cliniques de chronothérapie ont aussi fondé le développement d'une stratégie médico-chirurgicale agressive et efficace dans le cancer colorectal métastatique, et de rapporter les plus longues observations montrant la possibilité de guérisons dans cette pathologie précédemment considérée comme incurable (Bismuth, Adam et al. 1996; Bismuth and Adam 1998; Giacchetti, Itzhaki et al. 1999; Adam, Avisar et al. 2001; Adam, Delvart et al. 2004; Adam 2007; Adam, Aloia et al. 2007; Adam,

Wicherts et al. 2008; de Haas, Wicherts et al. 2008; Adam, Wicherts et al. 2009; Levi, Bouchahda et al. 2010).

Mélatonine
Ramelteon
Tasimelteon
Agomélanine
LY156735
Lithium
ACTH
Triazolam
Caféine
L-DOPA
Gepirone
Fluoxetine
Imipramine
SB-649868

II.5.4. Effet chronobiotique des agents anticancéreux

Chronobiotique se dit de toute substance, y compris de nombreux médicaments utilisés pour d'autres indications thérapeutiques, qui influencent et modifient la fonction du système circadien, et donc au moins un rythme biologique.

Au-delà des agents chronobiotiques classiques, tels que la mélatonine, les glucocorticoïdes ou d'autres médicaments agissant sur le système nerveux central (Tableau VI), plusieurs autres

Tableau VI. Agents pharmacologiques avec activité chronobiotique démontrée chez l'Humain.

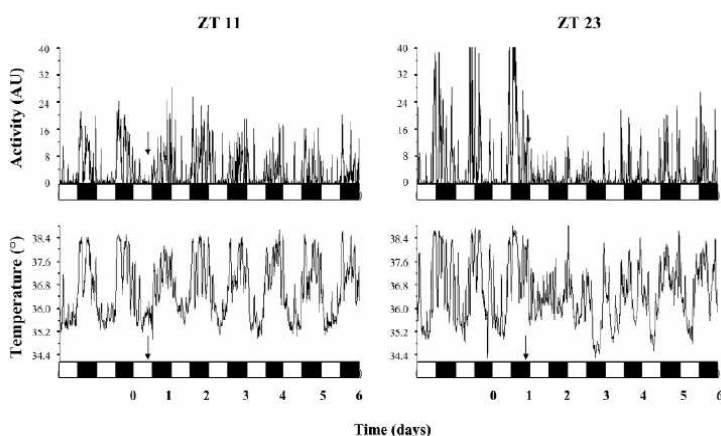
médicaments utilisés en cancérologie, et plus particulièrement les agents cytotoxiques antitumoraux, ont la capacité d'altérer la fonction du système

circadien : ils possèdent donc une activité chronobiotique.

Ces effets méconnus jusque récemment, se fondent sur la démonstration expérimentale d'une disruption circadienne chimio-induite, qui touche deux composants du système circadien, les rythmes de la physiologie circadienne et l'horloge moléculaire. Les données cliniques sont encore limitées. Elles montrent toutefois une claire perturbation des rythmes circadiens des patients à la suite de l'administration d'un cycle de chimiothérapie (Berger

Figure 19. Exemple d'effet d'un agent cytotoxique anticancéreux sur la physiologie circadienne selon son horaire d'administration chez la Souris.

Perturbation des rythmes circadiens de l'activité locomotrice (en haut) et de la température corporelle centrale (en bas) des souris, induite par l'administration de Gemcitabine IV. On note une perturbation plus importante des deux rythmes par l'administration de ce médicament à ZT 23 [Hours After Light Onset, HALO] (fin de phase d'obscurité) alors que celle-ci est moindre après injection à ZT 11 (fin de phase de lumière). L'administration de la Gemcitabine à ZT 11 est ainsi associée à la moindre perte de poids des souris et à la plus grande inhibition tumorale. D'après (Li and Levi 2007).



and Higginbotham 2000; Roscoe, Morrow et al. 2002; Fouladiun, Korner et al. 2007; Iurisci, Rich et al. 2007; Savard, Liu et al. 2009; Berger, Grem et al. 2010), et font ressortir la disruption circadienne comme une nouvelle toxicité de la chimiothérapie.

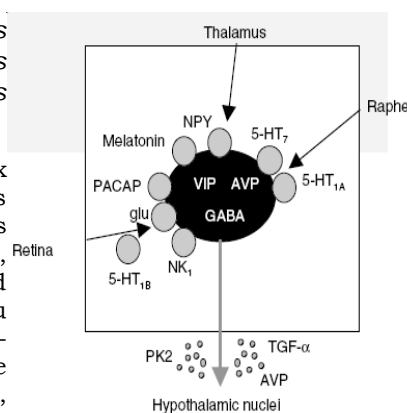
II.5.4.1. Effet sur la physiologie circadienne

La perturbation du système circadien de la souris par l'administration d'un agent anticancéreux cytotoxique tel que la vinorelbine, la gemcitabine ou l'irinotecan, ou d'un immunomodulateur tel que l'interferon-gamma, a été rapportée pour les rythmes circadiens d'activité locomotrice et de température péritonéale (Ohdo, Koyanagi et al. 2001; Li, Kanekal et al. 2006; Li and Levi 2007; Ahowesso, Li et al. 2011) (Figure 24). L'importance de la disruption transitoire et spontanément réversible du médicament dépend de l'horaire d'administration. Elle est plus sévère, voire exclusivement présente après administration au stade circadien de toxicité hématologique et/ou digestive et/ou rénale maximale (Ohdo, Koyanagi et al. 2001; Li, Kanekal et al. 2006; Li and Levi 2007; Ahowesso, Li et al. 2011). Les mécanismes précis d'une telle disruption demeurent indéterminés, et peuvent impliquer le pacemaker hypothalamique, ses voies de signalisation et/ou les horloges périphériques moléculaires, ainsi que les mécanismes de leur entrainement circadien.

Chez l'Homme, la situation se complique à cause de deux facteurs : la présence du cancer, lui-même associé à une perturbation de la fonction circadienne (cf. supra, chapitre II.4.2.1), et l'utilisation usuelle de médicaments de support, en association à la chimiothérapie. Parmi les médicaments de support, relevons les glucocorticoïdes, à visée antiallergique et/ou antiémétique. De plus, le pacemaker

Figure 20. Récepteurs connus des neuromédiateurs sur les neurones des noyaux suprachiasmatiques des Mammifères.

Schéma des circuits neuronaux des noyaux suprachiasmatiques (NSC), avec les neuromédiateurs intrinsèques aux neurones des NSC (Vasoactive Intestinal Polypeptide [VIP], Arginin Vasopressin [AVP], γ -Aminobutyric Acid [GABA]), en provenance d'autres structures du système nerveux (serotonine [5-HT], Neurokinin-1 [NK-1], glutamate [glu], Pituitary Adenylate Cyclase-Activating Peptide [PACAP], Neuropeptide Y [NPY]), et de sortie des NSC (Prokineticin-2 [PK-2], Transforming Growth Factor- α [TGF- α]). D'après (Sprouse 2004).



hypothalamique, avec sa fonction d'intégration des informations sur l'environnement extérieur cyclique, possède des

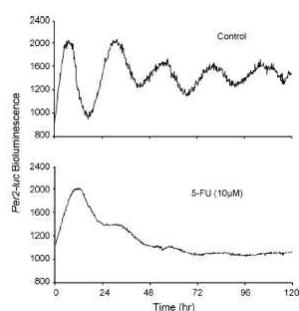


Figure 21. Exemple de perturbation de l'horloge circadienne moléculaire par un agent cytotoxique anticancéreux.

Profils de bioluminescence des cellules C6 de rat porteuses d'un gène de fusion du promoteur de Per2 avec la luciférase, synchronisées par la dexaméthasone en présence ou non de 5-Fluorouracile (10 µM). Cet antimétabolite provoque une perturbation importante des oscillations physiologiques de l'horloge circadienne moléculaire des cellules. D'après (Terazono, Hamdan et al. 2008).

récepteurs pour plusieurs neuromédiateurs, et envoi des fibres neuronales afférentes vers d'autres noyaux du système nerveux central (Buijs and Kalsbeek 2001; Sprouse 2004; Siepka, Yoo et al. 2007; Takahashi, Hong et al. 2008; Dibner, Schibler et al. 2010; Welsh, Takahashi et al. 2010). Certains médicaments de support ont ainsi un effet sur les récepteurs des neuromédiateurs solubles ou synaptiques. Ils pourraient au plan théorique altérer la coordination centrale circadienne (Figure 25). Ce possible effet chronobiotique des médicaments de support associés à la chimiothérapie reste à explorer chez l'animal et chez l'Homme.

II.5.4.2. Effet sur l'horloge moléculaire

La perturbation chimio-induite du système circadien ne se limite pas à de la physiologie circadienne, mais elle concerne aussi l'horloge circadienne moléculaire. L'altération

temporaire des oscillations des gènes de l'horloge dans les NSC, le foie et la glande surrénale a été démontrée après administration d'interféron-gamma chez la Souris (Ohdo, Wang et al. 2000; Ohdo, Koyanagi et al. 2001). De même, la survenue de lésions de l'ADN consécutives aux radiations ionisantes ou à des agents chimiques, peut modifier la phase des rythmes endogènes (Oklejewicz, Destici et al. 2008;

Molécule	Cible principale	Phénotype de la période	Type de cellule utilisé
IC261	CKIδ/ε	Longue	Rat-1
CKI-7	CKIδ/ε	Longue	NIH3T3
D4476	CKIδ/ε	Longue	NIH3T3
PF-670462	CKIδ/ε	Longue	NIH3T3
PF-4800567	CKIε	Inchangé	Rat-1
DRB	CK2	Longue	<i>Aplysia</i>
DMAT	CK2	Longue	U2OS ; NIH3T3
Indirubin -3'-oxime	CDK ; GSK-3	Pas testé	
Kenpaullone	CDK ; GSK-3	Court	Rat-1
Chir99021	GSK-3	Pas testé	
1-azakenpaullone	GSK-3	Pas testé	
Indirubin derivatives	GSK-3	Court	Rat-1
Roscovitine	CDK	Longue	<i>Bulla</i>
SB203580/SB202190	p38	Longue	Glande pinéale de poulet
SP600125	JNK	Longue	Noyau suprachiasmatique de souris

Tableau VII. Liste des inhibiteurs de kinases qui changent la période circadienne *in vitro*.

Modifiée d'après (Hirota and Kay 2009).

Destici, Oklejewicz et al. 2009). Dans le modèle cellulaire, l'exposition des cellules au 5-fluorouracile a provoqué une disruption importante de l'horloge moléculaire (Terazono, Hamdan et al. 2008) (Figure 26), témoignant d'un effet direct du médicament sur l'horloge circadienne.

Plusieurs molécules, médicaments commercialisés ou en voie d'expérimentation, peuvent modifier la période ou de la phase de l'horloge circadienne moléculaire *in vitro*, par un effet direct inhibiteur des cibles protéiques impliquées dans le contrôle des oscillations moléculaires circadiennes (Tableau VII).

L'analyse critique exhaustive de la littérature scientifique relative à la chronothérapie des cancers fait l'objet de deux revues, l'une fondamentale, l'autre clinique, présentées dans la section Résultats (cf. articles #1 et #2) (Innominato, Levi et al. 2010; Levi, Okyar et al. 2010).

III. PROBLEMATIQUE ET OBJECTIFS

III.1. Problématique et Contexte

III.1.1. Système circadien, cancers et chronothérapeutique

L'organisation hiérarchique du système circadien intervient dans le contrôle temporel de la physiologie humaine à l'échelle des organes et de l'organisme entier (cf chapitre II.2) (Panda, Hogenesch et al. 2002; Reppert and Weaver 2002; Hastings, Reddy et al. 2003; Gachon, Nagoshi et al. 2004; Lévi and Schibler 2007; Liu, Lewis et al. 2007; Reddy and O'Neill 2010). La découverte, dans les années 1950-60, des rythmes circadiens humains, en particulier pour la sécrétion de cortisol, le comportement, et la tension artérielle ont donné lieu à plusieurs applications médicales notamment en endocrinologie, en psychiatrie et en cardiologie (Roenneberg and Merrow 2005; Koukkari and Sothorn 2006; Haus 2007; Lemmer 2007; Lévi and Schibler 2007; Smolensky and Peppas 2007; Lemmer 2009; Reddy and O'Neill 2010). La découverte, en 1995-2000, d'une horloge moléculaire ubiquitaire chez les mammifères, a permis d'améliorer de façon critique la compréhension des mécanismes du contrôle circadien de la physiologie à l'échelle cellulaire, et leur intégration aux échelles tissulaire et organique, ainsi qu'au niveau de l'organisme entier (Roenneberg and Merrow 2005; Koukkari and Sothorn 2006; Lévi and Schibler 2007; Lemmer 2009; Reddy and O'Neill 2010). Ces approfondissements ont particulièrement concerné les relations entre l'horloge circadienne et le cycle cellulaire, servant ainsi de base au développement d'applications thérapeutiques en oncologie (Chen-Goodspeed and Lee 2007; Gery and Koeffler 2007; Levi, Filipinski et al. 2007). Ainsi, les expérimentations de chronothérapie ont été d'abord fondées sur l'observation des cycles biologiques circadiens, auxquels ont été ajustées les administrations des médicaments anticancéreux (Lévi 2001; Levi 2002; Mormont and Levi 2003; Levi 2006).

Mon travail de thèse s'inscrit dans le cadre des recherches concernant le rôle du système circadien dans le développement et la progression des cancers, dans la perspective de nouvelles options thérapeutiques. Un aspect important concerne le rôle de la disruption du système circadien dans l'apparition de symptômes généraux ou spécifiques d'un ou plusieurs organes, dont la fonction est par ailleurs régulée par le système circadien. Rappelons que la désynchronisation de l'horloge

circadienne centrale par rapport aux cycles de 24 h de l'environnement externe, suite à un voyage transmérien rapide, provoque souvent une sensation de désorientation spatio-temporelle et des symptômes associés fréquemment, regroupés sous le terme de « décalage horaire » (Waterhouse, Reilly et al. 1997; Waterhouse, Reilly et al. 2007; Sack 2010). Les symptômes du décalage horaire incluent fatigue, anorexie, céphalée, troubles du sommeil et de l'humeur, somnolence diurne, malaise général, perte de concentration intellectuelle, irritabilité, et/ou troubles du transit (Waterhouse, Reilly et al. 1997; Waterhouse, Reilly et al. 2007; Sack 2010). Ces symptômes régressent spontanément en quelques jours, au fur et mesure que l'horloge interne se synchronise au nouvel environnement photopériodique et social. La vitesse de resynchronisation varie selon le sujet, la direction du vol transmérien et le nombre de fuseaux horaires traversés (Waterhouse, Reilly et al. 1997; Waterhouse, Reilly et al. 2007; Sack 2010).

III.1.2. Cancers et symptômes

Les patients atteints de cancer présentent souvent des symptômes généraux, quelle que soit la localisation et le type de tumeur primitive. La survenue de ces symptômes fait pratiquer les examens et conduit ainsi souvent à découvrir le cancer. Ces symptômes reflètent aussi l'évolutivité de la maladie cancéreuse. Il a été rapporté que 84% des patients se plaignent d'au moins un symptôme qui altère leur qualité de vie, avec une médiane de 11 sur 25 symptômes explorés par patient (Walsh and Rybicki 2006) . L'évaluation systématique du nombre et de la sévérité des symptômes rapportés par les patients cancéreux est effectuée à l'aide de questionnaires subjectifs, qui ont fait l'objet de validations, d'une part interne (stabilité, test-retest, pertinence, variation temporelle), d'autre part internationale (multilingue) (Aaronson, Ahmedzai et al. 1993; Cocks, King et al. 2008). Malgré un développement avec validation en quatre phases (identification des problèmes, construction de la liste des questions, pre-test, test sur le terrain), ces questionnaires laissent persister des doutes quant à leur précision en raison de leur subjectivité. Dans les études d'incidence de symptômes à l'aide de questionnaires remplis par le patient, la douleur, la fatigue, l'anorexie, le manque d'énergie et la constipation, représentent les symptômes les plus fréquemment rapportés par les patients atteints de cancer métastatique (Cleeland, Mendoza et al. 2000; Barsevick, Whitmer et al. 2006; Walsh and Rybicki 2006; Cheung, Le et al. 2009; Esper 2010;

Xiao 2010). Non seulement plusieurs symptômes sont, en règle, associés chez le même patient, mais souvent les mêmes symptômes cliniques sont, en règle, associés chez un même patient, d'où le concept de « groupes de symptômes » (« symptom clusters ») (Molassiotis, Wengstrom et al. ; Skerman, Yates et al. ; 2002; Beck 2004; Dodd, Miaskowski et al. 2004; Fleishman 2004; Lee, Dantzer et al. 2004; Miaskowski, Dodd et al. 2004; Paice 2004; Barsevick, Whitmer et al. 2006; Chen and Tseng 2006; Miaskowski 2006; Walsh and Rybicki 2006; Miaskowski, Aouizerat et al. 2007; Rich 2007; Kim, Barsevick et al. 2008; Pud, Ben Ami et al. 2008; Cheung, Le et al. 2009; Liu, Fiorentino et al. 2009; Ott, Ullrich et al. 2009; Yamagishi, Morita et al. 2009). Le concept de « groupe de symptômes » ne dérive pas uniquement d'analyses statistiques montrant diverses associations de symptômes, mais aussi de la clinique. Ainsi il a été proposé une étiologie spécifique de chaque groupe de symptômes (Molassiotis, Wengstrom et al. ; Skerman, Yates et al. ; 2002; Beck 2004; Dodd, Miaskowski et al. 2004; Fleishman 2004; Lee, Dantzer et al. 2004; Miaskowski, Dodd et al. 2004; Paice 2004; Barsevick, Whitmer et al. 2006; Chen and Tseng 2006; Miaskowski 2006; Walsh and Rybicki 2006; Miaskowski, Aouizerat et al. 2007; Rich 2007; Kim, Barsevick et al. 2008; Pud, Ben Ami et al. 2008; Cheung, Le et al. 2009; Liu, Fiorentino et al. 2009; Ott, Ullrich et al. 2009; Yamagishi, Morita et al. 2009). Selon plusieurs équipes les groupes de symptômes les plus fréquemment retrouvés chez les patients atteints de cancer présentent une grande stabilité longitudinale quel que soit le cancer dont ils souffrent (Dodd, Miaskowski et al. 2001; Miaskowski, Dodd et al. 2004; Olson, Hayduk et al. 2008; Kim, Jahan et al. 2009; Kim, Jahan et al. 2009; Liu, Fiorentino et al. 2009; Yamagishi, Morita et al. 2009; Kirkova, Walsh et al. 2010; Molassiotis, Wengstrom et al. 2010; Xiao 2010; Skerman, Yates et al. 2011). L'association de fatigue (aussi dénommée asthénie) et d'anorexie, parfois complétée par la douleur, les troubles du sommeil et/ou dépression, forme le groupe de symptômes le plus souvent retrouvé dans les études d'incidence et d'association des symptômes (Cleeland, Mendoza et al. 2000; Barsevick, Whitmer et al. 2006; Walsh and Rybicki 2006; Rich 2007; Aprile, Ramoni et al. 2008; Cheung, Lee et al. 2009; Esper 2010; Xiao 2010).

Il n'existe aucun traitement vraiment efficace de la fatigue et de l'anorexie des patients cancéreux, malgré les recherches actives et de longue date (2002; Yavuzsen, Davis et al. 2005; Dy, Lorenz et al. 2008; Minton, Richardson et al. 2008). Ces symptômes peuvent non seulement refléter une maladie cancéreuse, mais aussi être provoqués par les traitements anticancéreux. Ainsi, l'identification

des mécanismes physiopathologiques du groupe de symptômes associant fatigue et anorexie pourrait-elle ouvrir la voie à des stratégies thérapeutiques nouvelles et innovantes en cancérologie.

III.1.3. Chimiothérapie et symptômes

Le développement fondamental et clinique de nouveaux agents anticancéreux, et l'optimisation de leurs schémas d'administration rendent compte de l'amélioration lente mais régulière de la survie des patients cancéreux, et ce malgré l'augmentation d'incidence des néoplasies (La Vecchia, Bosetti et al. 2010; Bosetti, Levi et al. 2011; Jemal, Bray et al. 2011). Le cancer colorectal métastatique constitue un paradigme de cette tendance : en effet, la survie médiane des nouveaux patients est passée de ~10 mois en 1990 à ~ 16 mois, dans la décennie suivante, pour atteindre ~ 20 mois actuellement (Segal and Saltz 2009; La Vecchia, Bosetti et al. 2010; Bosetti, Levi et al. 2011). Les données de survie de ces patients sont souvent encore meilleures dans les centres spécialisés qui ont mis en place des stratégies multidisciplinaires agressives, et plusieurs cas rares mais non anecdotiques de guérison ont été rapportés en particulier dans notre Unité (Adam, Wicherts et al. 2009). L'amélioration de l'efficacité des traitements médicaux conventionnels est obtenue cependant au prix d'une toxicité tissulaire et générale importante (Fortner, Schwartzberg et al. 2007; Aprile, Ramoni et al. 2008; Eng 2009). En particulier, les symptômes toxiques provoqués par la chimiothérapie ou la radiothérapie sont tout à fait semblables à ceux liés au cancer, et comprennent en particulier fatigue, anorexie, perte de poids, troubles du sommeil, cachexie et troubles digestives (Fortner, Schwartzberg et al. 2007; Honea, Brant et al. 2007; Aprile, Ramoni et al. 2008; Aprile, Ramoni et al. 2009; Eng 2009; Kim, Jahan et al. 2009; Esper 2010; Molassiotis, Wengstrom et al. 2010; Xiao 2010). Plus particulièrement, le groupe « fatigue et anorexie » est une association de symptômes provoquée par la combinaison FOLFOX, un des schémas de chimiothérapie le plus utilisé contre le cancer colorectal qui combine 5-fluorouracile, leucovorine et oxaliplatine (Aprile, Ramoni et al. 2008; Aprile, Ramoni et al. 2009). Ces observations sont en faveur d'un mécanisme physiopathologique commun des symptômes de ce groupe, que j'étudie plus particulièrement dans ma thèse.

III.1.4. Chronothérapeutique des cancers

Les progrès de la chimiothérapie du cancer colorectal métastatique dans ces deux dernières décennies ont été pour partie fondés sur l'optimisation de l'horaire d'administration des agents anticancéreux (Tableau IV) (Lévi 2001; Giacchetti 2002; Levi 2002; Mormont and Levi 2003; Levi 2006). La chronothérapeutique des cancers humains se fonde sur les mécanismes circadiens du contrôle moléculaire du cycle de division cellulaire, de l'apoptose et de la réparation de l'ADN et sur les nombreuses validations expérimentales (cf. chapitre II.5) (Hrushesky and Bjarnason 1993; Fu and Lee 2003; Hrushesky, Wood et al. 2004; Levi 2006; Levi, Filipski et al. 2007; Lévi and Schibler 2007; Wood, Yang et al. 2009). Ces recherches ont permis, dès 1990, d'améliorer l'index thérapeutique de l'association de 5-fluorouracile, de leucovorine et d'oxaliplatine dans le cancer colorectal métastatique (cf chapitre II.5.3) (Tableau IV) (Caussanel, Lévi et al. 1990; Lévi, Misset et al. 1992; Levi, Perpoint et al. 1993; Lévi, Zidani et al. 1994; Bertheault-Cvitkovic, Jami et al. 1996; Garufi, Levi et al. 1997; Lévi, Zidani et al. 1997; Lévi, Zidani et al. 1999; Giacchetti, Perpoint et al. 2000; Cure, Chevalier et al. 2002; Levi, Focan et al. 2007). Cependant, l'optimisation simultanée de la tolérance et de l'efficacité gagnerait encore à une personnalisation de l'administration chronothérapeutique (Lévi and Schibler 2007). Le développement de telles méthodes doit prendre en compte l'impact clinique des symptômes généraux, qui affectent plusieurs domaines de la qualité de vie (Dodd, Miaskowski et al. 2001; Jordhoy, Fayers et al. 2001; Miaskowski, Cooper et al. 2006; Buchanan, O'Mara et al. 2007; Miaskowski, Aouizerat et al. 2007; Ferreira, Kimura et al. 2008; Pud, Ben Ami et al. 2008; Liu, Fiorentino et al. 2009; Esper 2010; Xiao 2010; Martinelli, Quinten et al. 2011). En effet, ces symptômes peuvent affecter altérer la sensation de bien-être du patient, et détériorer ses fonctions physiques, sociales et mentales (Dodd, Miaskowski et al. 2001; Jordhoy, Fayers et al. 2001; Miaskowski, Cooper et al. 2006; Buchanan, O'Mara et al. 2007; Miaskowski, Aouizerat et al. 2007; Ferreira, Kimura et al. 2008; Pud, Ben Ami et al. 2008; Liu, Fiorentino et al. 2009; Esper 2010; Xiao 2010; Martinelli, Quinten et al. 2011). Bien que rare et imprévisible, la guérison de cancer colorectal métastatique est possible. Cependant, la plupart des traitements de cette affection vise à augmenter le taux de rémissions complètes durables, dans le cadre de stratégies médico-chirurgicales personnalisées, et, au moins, à prolonger la survie des patients - ajouter des années à la vie -, tout en préservant voire améliorant la qualité de vie - ajouter de la vie aux années.

III.2. Hypothèses et Objectifs

Les recherches réalisées dans le cadre de cette thèse se fondent sur l'hypothèse d'un rôle important du système circadien dans les processus cancéreux et leurs traitements. De ce fait, l'altération du système circadien, au niveau de sa coordination centrale, de sa physiologie circadienne et/ou de ses horloges moléculaires dans les organes périphériques peut accélérer la progression cancéreuse et raccourcir la survie des patients, hypothèses fondées sur le modèle expérimental. D'autre part, le groupe de symptômes « fatigue-anorexie » est le plus fréquemment rapporté par les patients atteints de cancer, mais aussi par les sujets intolérants au décalage horaire provoqué par les vols transmériidiens longs courriers ou par le travail posté ou de nuit (Waterhouse, Reilly et al. 1997; Drake, Roehrs et al. 2004; Foster and Wulff 2005; Reinberg, Ashkenazi et al. 2007; Waterhouse, Reilly et al. 2007). Dans ces deux conditions, les sujets présentent une altération profonde et transitoire du système circadien, caractérisé par une désynchronisation interne (cf chapitres II.1, II.2 et II.4.2.2).

Nous formulons l'hypothèse que la fatigue et l'anorexie des patients cancéreux, sous traitement ou non, résulte d'une disruption circadienne centrale en rapport avec l'exposition du pacemaker hypothalamique à plusieurs cytokines pro-inflammatoires, telles que le TGF α , l'IL6 et, à un moindre degré, le TNF α . Dans un travail précédent, j'avais montré l'existence d'une disruption du rythme d'activité-repos chez les patients présentant une élévation des concentrations sériques de ces trois cytokines (Rich, Innominato et al. 2005). Ainsi le maintien ou la restauration d'un système circadien fonctionnel serait-il indiqué par une moindre incidence et une moindre sévérité des symptômes en rapport avec une disruption circadienne.

Les objectifs généraux de cette thèse comprennent :

- a) La définition des relations entre symptômes généraux et système circadien des patients atteints de cancer avant et pendant chimiothérapie. J'ai particulièrement examiné le groupe associant fatigue et anorexie chez les patients atteints de cancer colorectal métastatique.
- b) La quantification de l'impact clinique de la disruption circadienne sur la qualité de vie et sur la survie des patients atteints de cancer colorectal métastatique. J'ai examiné celui-ci avant et pendant l'administration d'une chimiothérapie conventionnelle ou chronomodulée.

Les objectifs spécifiques incluent :

- 1) l'évaluation critique de la littérature concernant la chronothérapeutique des cancers ;
- 2) la description de l'effet de la chimiothérapie, chronomodulée ou non, sur le système circadien des patients;
- 3) l'identification d'une mesure quantitative de la fonction circadienne au cours de la chimiothérapie, indispensable tant pour une détection précoce que pour évaluer l'efficacité des interventions personnalisées visant à préserver ou restaurer le système circadien
- 4) la définition d'un index quantitatif de disruption circadienne cliniquement pertinent, pour la survie globale ;
- 5) l'identification des facteurs principaux qui modifient l'activité antitumorale d'un schéma fixe de chronothérapie fondé sur l'expérimentation animale réalisée chez des souris mâles, à savoir :
 - le système circadien, estimé d'après le rythme d'activité-repos
 - la tolérance à la chimiothérapie
 - le sexe

Ce travail de thèse vise ainsi à faire émerger des propositions thérapeutiques nouvelles ciblant le système circadien qui puissent prévenir ou améliorer les symptômes généraux des patients atteints de cancer avancé.

Le système circadien des patients cancéreux pourrait être préservé ou renforcé par l'optimisation circadienne personnalisée de la chimiothérapie, par l'utilisation de médicaments dits « chronobiotiques », et/ou par des interventions comportementales.

IV. CONDUITE ET METHODOLOGIE GENERALES DU PROJET

Ce projet de thèse a abordé cette problématique autour du rôle du système circadien dans la pathogénèse de plusieurs symptômes, tels que la fatigue et l'anorexie, du point de vue de son impact clinique et d'ouverture à des thérapeutiques innovantes.

IV.1. Relations entre système circadien et symptômes avant et pendant chimiothérapie

J'ai d'abord exploré les relations entre le rythme circadien d'activité et repos, considéré ici comme un biomarqueur du « pacemaker » central du système circadien (cf chapitre II.3.1) d'une part, et les symptômes, les fonctions et les domaines de la qualité de vie d'autre part. Cette première étude concerne 252 patients atteints de cancer colorectal métastatique, avant administration de chimiothérapie. Elle a été réalisée à l'hôpital Paul Brousse et dans huit centres de cancérologie européens ou canadien. Le questionnaire EORTC QLQ C-30 v2.0 rempli par les patients a permis l'évaluation des domaines et fonctions de la qualité de vie et des symptômes (Aaronson, Ahmedzai et al. 1993). Le questionnaire EORTC-QLQ-C30 a été choisi, car son contenu et sa structure sont validés et fiables, et il est disponible en 82 langues. Il permet de mesurer en termes de sévérité le ressenti subjectif du patient vis-à-vis de 5 fonctions, de 9 symptômes et d'une échelle de qualité de vie globale (Aaronson, Ahmedzai et al. 1993). Pour ces raisons, ce questionnaire de qualité de vie est le plus largement utilisé en recherche clinique dans le monde (> 3000 essais cliniques).

La survenue d'une disruption circadienne a déjà été associée au groupe de symptômes d'intérêt chez des sujets intolérants au décalage horaire ou au travail posté (Waterhouse, Reilly et al. 1997; Drake, Roehrs et al. 2004; Reid, Chang et al. 2004; Foster and Wulff 2005; Waterhouse, Reilly et al. 2007; Reid and Zee 2009). Chez les patients cancéreux, la disruption circadienne a d'abord été corrélée à la fatigue par le laboratoire RBC. Il s'agissait d'une cohorte de patients atteints de cancer colorectal métastatique, dont la majorité avait reçu une chimiothérapie antérieure (Mormont, Waterhouse et al. 2000; Mormont and Waterhouse 2002). Une telle corrélation a été ensuite confirmée par plusieurs équipes dans le monde chez des patients atteints de divers cancers (Roscoe, Morrow et al. 2002; Fouladiun, Korner et al. 2007; Miller, Ancoli-Israel et al. 2008; Berger, Wielgus et al. 2009; Liu, Fiorentino et al. 2009; Payne 2011). Néanmoins, seule l'étude initiale a précisé l'impact clinique global d'une disruption circadienne vis-à-vis de différents

symptômes et de la qualité de vie des patients, objet de la thèse de Doctorat de M.C. Mormont (Mormont, Waterhouse et al. 2000; Mormont and Waterhouse 2002).

J'ai ensuite examiné les associations entre disruption circadienne induite par la chimiothérapie, symptômes et toxicités cliniques du traitement, gradées par le médecin à l'aide d'une échelle internationale validée et utilisée dans tous les essais cliniques (NCI-CTC-AE v2.0). La fonction circadienne a été évaluée à partir d'un enregistrement continu du rythme d'activité-repos pendant 72 heures au cours de la semaine suivant le début d'un cycle de chimiothérapie chronomodulée ou conventionnelle chez 77 patients. J'ai systématiquement considéré les principales toxicités cliniques, mais j'ai plus particulièrement examiné la fatigue et la perte de poids provoquée par la chimiothérapie, compte tenu des résultats déjà obtenus expérimentalement, et chez les patients, avant traitement (Ohdo, Makinosumi et al. 1997; Mormont, Waterhouse et al. 2000; Mormont and Waterhouse 2002; Filipski, Lemaigre et al. 2004; Fouladiun, Korner et al. 2007; Li and Levi 2007; Hrushesky, Grutsch et al. 2009; Ahowesso, LI et al. 2011; Payne 2011) (cf. article #6).

Ces études situent le rôle du système circadien dans l'apparition des symptômes et dans la qualité de vie des patients cancéreux, en l'absence de traitement ou pendant son administration.

IV.2. Evaluation de l'impact clinique de la disruption circadienne sur la qualité de vie et sur la survie des patients cancéreux

J'ai ensuite évalué l'impact d'une altération du système circadien avant traitement sur l'efficacité de la chimiothérapie et le devenir du patient. Cette recherche a été motivée par les résultats expérimentaux montrant une croissance tumorale accrue chez la Souris présentant une disruption anatomique ou fonctionnelle du pacemaker circadien central (Filipski, King et al. 2002; Filipski, Delaunay et al. 2004; Filipski, Innominato et al. 2005; Filipski, Li et al. 2006; Filipski and Levi 2009), et par deux études montrant une valeur pronostique négative de la disruption circadienne sur la survie des patients atteints de cancer avancé indépendamment des facteurs pronostiques connus (Mormont, Waterhouse et al. 2000; Sephton, Sapolsky et al. 2000) (cf. chapitre II.4.1.2). Les résultats expérimentaux et cliniques princeps ont été générés indépendamment dans le laboratoire RBC (F. Lévi) et dans l'Université de Stanford (D. Spiegel).

Avant d'entreprendre cette thèse, j'ai participé à deux études sur le rôle du système circadien dans la progression cancéreuse, l'une expérimentale et l'autre translationnelle. Nous avons mis en évidence l'accélération de la croissance

tumorale par le décalage horaire chronique et son ralentissement par une prise alimentaire circadienne programmée chez la Souris, ainsi que plusieurs mécanismes moléculaires impliqués (Filipski, Innominato et al. 2005). Chez 80 patients atteints de cancer colorectal métastatique, nous avons montré que les concentrations circulantes élevées de cytokines pro-inflammatoires telles que le TFG- α , le TNF- α et l'IL-6, étaient associées à une disruption circadienne et à une survie plus courte, confirmant l'hypothèse fondée sur le rôle de ces cytokines dans la coordination circadienne dans le modèle animal (Rich, Innominato et al. 2005). Ces deux études ouvrent des perspectives chronothérapeutiques nouvelles, notamment une resynchronisation des horloges circadiennes par l'alimentation programmée et l'utilisation d'inhibiteurs des récepteurs des cytokines étudiées, par exemple du récepteur à l'EGF.

Pour étudier l'impact d'une altération du système circadien sur l'efficacité de la chimiothérapie et le devenir du patient, j'ai eu accès à l'ensemble des données cliniques de l'étude translationnelle annexe à l'essai clinique randomisé contrôlé international de Phase III EORTC 05963 (Giacchetti, Bjarnason et al. 2006), auquel j'avais participé en tant que co-investigateur, lors de mon internat de cancérologie à l'Université « G. d'Annunzio » de Chieti (S. Iacobelli).

Afin de situer dans un contexte plus large, les relations entre disruption circadienne, symptômes, qualité de vie et survie des patients atteints de cancer colorectal métastatique avant tout traitement médical, j'ai étudié la valeur pronostique des symptômes et de la qualité de vie des patients pour la survie globale, sur une cohorte plus large de 443 patients inscrits dans l'essai EORTC 05963 en coopération avec le groupe Qualité de Vie de l'EORTC (Efficace, Bottomley et al. 2006; Efficace, Osoba et al. 2007; Gotay, Kawamoto et al. 2008).

IV.3. Effet de la chimiothérapie sur le système circadien et identification d'un critère objectif d'évaluation de la fonction circadienne

J'ai alors exploré l'effet de la chimiothérapie sur le système circadien des patients cancéreux au cours de son administration. Les données expérimentales ont en effet démontré que les agents cytotoxiques peuvent altérer, de façon transitoire mais profonde et dépendante du stade circadien d'administration, la physiologie circadienne et l'horloge moléculaire chez la Souris ou le Rat (Ohdo, Wang et al. 2000; Ohdo, Koyanagi et al. 2001; Li, Kanekal et al. 2006; Li and Levi 2007; Ahowesso, LI et al. 2011) (cf. chapitre II.5.4.1).

Le rythme d'activité et repos a été choisi comme biomarqueur du système circadien, en raison du caractère non invasif des mesures, nécessairement prolongées dans ce contexte (cf. chapitre II.3.1).

Dans une première étape, j'ai recherché les facteurs démographiques et les toxicités cliniques associées à une perturbation circadienne provoquée par la chimiothérapie chez 77 patients, dont le rythme d'activité-repos avait été enregistré au cours de la semaine suivant le début d'un cycle de traitement (cf. supra). J'ai limité l'intervalle d'étude à une semaine, car une étude indépendante montrait une récupération fréquente du système circadien dès la deuxième semaine, chez 95 patientes recevant une chimiothérapie adjuvante pour cancer mammaire (Savard, Liu et al. 2009). Cependant l'impact clinique des altérations transitoires de la physiologie circadienne n'était pas précisée (Savard, Liu et al. 2009). Une telle disruption circadienne a par ailleurs été obtenue par l'administration d'une douzaine d'agents anticancéreux selon leur dose et l'heure d'administration (Ohdo, Wang et al. 2000; Ohdo, Koyanagi et al. 2001; Li, Kanekal et al. 2006; Li and Levi 2007; Ahowesso, LI et al. 2011). Ici, j'ai cherché à identifier les facteurs associés à la survenue d'une disruption circadienne en cours de chimiothérapie, et son impact clinique sur la survie des patients (cf. article #8).

J'ai ensuite cherché à compléter les informations sur le système circadien, fournies par l'enregistrement du rythme d'activité-repos, par l'étude concomitante du rythme de la température corporelle, autre biomarqueur circadien robuste (cf. chapitres II.3.2 et II.3.5). La méthodologie nécessaire à cette approche a été mise au point en collaboration avec le Dr. A. Gorbach (National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Etats Unis), avec le soutien du Réseau d'Excellence Européen BIOSIM (FP6). Cette recherche a impliqué les mesures simultanées des rythmes d'activité-repos et de température de surface cutanée chaque minute pendant 2 à 6 jours, avant et/ou pendant l'administration de chronothérapie, après repérage des zones de mesure cutanées par caméra infrarouge chez 9 sujets (cf. article #7). Ce projet s'est ensuite développé et fait l'objet de la thèse de doctorat en cours de V. Roche, que j'encadre partiellement. Dans cette démarche, j'ai aussi collaboré à l'acquisition, l'analyse et l'interprétation des données d'enregistrement prolongé du rythme d'activité-repos avant, pendant et après administration de chimiothérapie chez 49 patients. Cette étude en cours est réalisée en collaboration avec E. Ortiz-Tudela, doctorante (Université de Murcia, Espagne), que j'encadre en partie.

IV.4. Définition d'un index quantitatif de disruption circadienne

J'ai cherché à définir l'index quantitatif de disruption circadienne qui présente la plus grande pertinence clinique, en prenant comme critère la réponse la survie globale. Le but de cette étude collaborative internationale, effectuée chez 130 patients traités dans 9 institutions en France, Italie, Belgique et Canada, était d'identifier le critère circadien le mieux approprié, mais aussi d'en établir le seuil quantitatif le mieux discriminant pour prévoir la survie. Le résultat fournit ainsi un appui important pour la réalisation d'études translationnelles ou interventionnelles futures. J'ai ensuite participé à la conception, l'organisation, la mise à jour et la réalisation d'une base de données appariant les données cliniques de 436 patients atteints de cancer colorectal métastatique ayant tous eu un enregistrement du rythme d'activité-repos pour confirmer l'importance clinique et translationnelle de l'enregistrement du rythme circadien d'activité-repos dans une base de données plus large, incluant aussi les patients traités hors protocole de recherche dans la pratique clinique courante. Ce travail est en cours de rédaction.

IV.5. Identification des facteurs qui influencent l'activité de la chronothérapie

L'enregistrement continu du rythme d'activité-repos par actimétrie du poignet pendant 72 heures avant le début de la chronothérapie chez 130 patients (cf. article #5) et pendant son administration chez 77 patients (cf. article #8) a permis d'évaluer le rôle pronostic de la disruption circadienne (cf. supra). L'impact clinique de l'altération du système circadien avant ou pendant traitement a été aussi recherché chez les patients recevant une chimiothérapie conventionnelle, dans le cadre de l'essai randomisé EORTC05963.

Afin de valider sur un plus grand nombre de patients les hypothèses formulées dans les études précédentes, j'ai exploré les relations entre tolérance hématologique et clinique de la chimiothérapie chronomodulée ou non, et son efficacité sur les données des 564 patients enregistrés dans l'essai EORTC 05963. En effet les relations entre chronotolérance et chronoefficacité font l'objet de nombreuses recherches aux plans expérimental et théorique (Altinok, Levi et al. 2009). J'ai exploré ces relations chez les patients atteints de cancer colorectal.

Pour explorer la conséquence de la toxicité de la chimiothérapie pour son efficacité antitumorale, j'ai d'abord cherché à confirmer la relation positive entre la neutropénie, qui limite la dose administrée, et l'efficacité de la chimiothérapie conventionnelle. Une telle relation avait été démontrée pour différents types de

cancer par plusieurs équipes. J'ai ensuite étudié cette relation pour un schéma fixe de chronothérapie. La mise en évidence d'une association robuste entre disruption circadienne et groupe de symptômes « fatigue-anorexie » m'a conduit à explorer la valeur pronostique de ce groupe de symptômes au cours du traitement. Afin de disposer d'une approche davantage quantitative de l'anorexie, j'ai pris comme critère la perte de poids, me fondant aussi sur l'abondante littérature liant perte de poids chimio-induite à disruption circadienne (Li, Kanekal et al. 2006; Fouladiun, Korner et al. 2007; Li and Levi 2007; Ahowesso, LI et al. 2011). J'ai ainsi testé l'hypothèse qu'une disruption circadienne induite par le traitement constituait un facteur de mauvais pronostic spécifique à la chronothérapie (cf. chapitre II.5.2.3).

Suite de la démonstration d’une importante différence d’efficacité et de tolérance de la chimiothérapie chronomodulée selon le sexe dans le cancer colorectal métastatique (Giacchetti, Bjarnason et al. 2006; Levi, Focan et al. 2007), et sur la base de données montrant des différences circadiennes liées au sexe tant chez l’Homme que chez les animaux de laboratoire (cf. chapitre II.2.6), j’ai participé à l’élaboration, la réalisation et l’interprétation de la première méta-analyse d’essais de chronothérapie, fondée sur les données individuelles (cf. article #9). Le but de ce

Problématique abordée	Article(s) #
Révision critique des principes, résultats et perspectives de la chronothérapeutique des cancers	1 ; 2 ; 3
Relations entre rythme circadien d'activité-repos et qualité de vie	5 ; 6
Valeur pronostique du rythme circadien d'activité-repos pour la survie	5
Valeur pronostique de la qualité de vie pour la survie	4
Effet de la chimiothérapie sur les rythmes circadiens	7 ; 8
Impact clinique de la disruption circadienne sous chimiothérapie	8
Valeurs pronostiques de la toxicité pour l’efficacité de la chimiothérapie conventionnelle et de la tolérance pour l’efficacité de la chronothérapie	10 ; 11
Rôle du sexe dans l'efficacité et la tolérance de la chronothérapie	9

Tableau VIII. Récapitulatif des articles de la thèse.

travail, dans le contexte de la thèse, était de confirmer la meilleure efficacité de la chimiothérapie chronomodulée en comparaison du traitement conventionnel chez les hommes atteints de cancer colorectal métastatique. Ceux-ci présentaient aussi une meilleure tolérance que les femmes à la chronothérapie. Etant donné qu'il s'agissait de trois essais randomisés de phase III (Lévi, Zidani et al. 1994; Lévi, Zidani et al. 1997; Giacchetti, Bjarnason et al. 2006), il était ainsi possible d'explorer la validité de cette corrélation dans le groupe de patients ayant reçu le traitement conventionnel, où l'horaire d'administration n'était pas fixe.

Enfin, j'ai préparé la mise en œuvre d'essais prospectifs visant à évaluer le bénéfice clinique en termes de symptômes et qualité de vie, d'une thérapeutique pharmacologique ou comportementale ciblée sur le système circadien des patients atteints de cancer (cf. chapitre VII).

V. RESULTATS

V.1. Révision critique des principes, résultats obtenus et perspectives de la chronothérapie des cancers

V.1.1. Article # 1, Annual Review of Pharmacology and Toxicology, 2010

Administration circadienne des traitements anticancéreux

Lévi F, Okyar A, Dulong S, Innominato PF, Clairambault J.

Le système circadien est composé d'horloges moléculaires, qui rythment sur 24 h. Le métabolisme et la détoxification des xénobiotiques, mais aussi plusieurs étapes du cycle cellulaire, de la réparation de l'ADN, de l'apoptose et de l'angiogenèse. Les horloges circadiennes cellulaires sont coordonnées par les rythmes physiologiques endogènes, de sorte qu'elles marquent la même heure dans les tissus de l'hôte qui constituent aussi les cibles de toxicité des médicaments anticancéreux. En conséquence, l'horaire d'administration peut modifier de 2 à 10 fois la tolérance des agents anticancéreux dans les modèles expérimentaux et chez les patients cancéreux. L'efficacité antitumorale est aussi améliorée lorsque les médicaments sont donnés en proximité de leurs horaires respectifs de meilleure tolérance, en raison: (a) du mauvais entraînement circadien intrinsèque des tumeurs et (b) de la persistance de l'entraînement circadien des tissus sains. Inversement, les horloges de l'hôte sont perturbées lorsque les médicaments anticancéreux sont administrés aux stades circadiens de toxicité maximale. Par ailleurs, la disruption du système circadien accélère la croissance cancéreuse expérimentale et clinique. Le sexe, la physiologie circadienne, les gènes de l'horloge, et le cycle cellulaire influencent de façon importante les résultats de la chronothérapie des cancers. Actuellement, des approches mathématiques et de biologie des systèmes développent et intègrent les outils théoriques, expérimentaux et technologiques qui permettent d'optimiser et de personnaliser l'administration circadienne des traitements contre le cancer.



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Circadian Timing in Cancer Treatments

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Key Words

molecular clock, drug delivery, chronotherapeutics, gender, mathematical models, clinical trial methodology, systems biology, personalized medicine

Abstract

The circadian timing system is composed of molecular clocks, which drive 24-h changes in xenobiotic metabolism and detoxification, cell cycle events, DNA repair, apoptosis, and angiogenesis. The cellular circadian clocks are coordinated by endogenous physiological rhythms, so that they tick in synchrony in the host tissues that can be damaged by anticancer agents. As a result, circadian timing can modify 2- to 10-fold the tolerability of anticancer medications in experimental models and in cancer patients. Improved efficacy is also seen when drugs are given near their respective times of best tolerability, due to (a) inherently poor circadian entrainment of tumors and (b) persistent circadian entrainment of healthy tissues. Conversely, host clocks are disrupted whenever anticancer drugs are administered at their most toxic time. On the other hand, circadian disruption accelerates experimental and clinical cancer processes. Gender, circadian physiology, clock genes, and cell cycle critically affect outcome on cancer chronotherapeutics. Mathematical and systems biology approaches currently develop and integrate theoretical, experimental, and technological tools in order to further optimize and personalize the circadian administration of cancer treatments.

UGT: UDP-glucuronosyl transferase

5-FU: 5-fluorouracil

Circadian: biological rhythm with an about one day period (circa, about; dies, day)

Chronotherapeutics: the administration of treatments according to circadian or other biological rhythms

Biological rhythm: self-sustained and endogenous biological oscillation

Period: cycle duration

Circadian timing system (CTS): the biological system that generates ~24 hour rhythms in cellular and organism physiology and adjusts them to environmental cycles

INTRODUCTION

The outcomes of patients receiving anticancer treatments remain complicated by unpredictable severe toxicities and/or poor antitumor efficacy (1). Greater than 10-fold interindividual changes in drug exposure and pharmacokinetic parameters as well as the variable status of gene expression and metabolism within the tumor itself likely contribute to large interpatient variability in therapeutic index (1, 2). Whereas mapping the genetic polymorphisms in drug metabolism and detoxification can predict undue drug toxicity, the identification of molecular signatures in tumor cells can predict efficacy of specific anticancer drugs (3, 4). Recent results, however, emphasize that the relevance of the UDP-glucuronosyl-transferase *Ugt1a1**28 polymorphism, an FDA-approved test for the prediction of irinotecan toxicity, varies according to gender, delivery schedule, drug dose level, and associated genetic polymorphisms (5). Similar findings are reported for the polymorphisms of *Dpyd*, which encodes for dihydropyrimidine dehydrogenase, the rate-limiting enzyme for the catabolism of 5-fluorouracil (5-FU) (6).

The large variability in the outcome of patients on anticancer therapy is paralleled by the limited success rate of anticancer drug development. Only 5% of the anticancer agents selected for clinical development successfully complete all clinical phases and become registered as medications. Poor prediction of safety is identified as the main cause for interrupted clinical development (7).

This review emphasizes that treatment timing within the 24-h timescale, that is, circadian (circa, about; dies, day) timing, can predictably change by severalfold the tolerability and the antitumor efficacy of anticancer agents both in experimental models and in cancer patients.

Indeed, most biological functions display circadian changes in mammals (8). The disruption of circadian clocks that drive these rhythms favors cancer processes and reduces survival in cancerous rodents and human patients (9–14). A recent monograph by the International Agency for Research on Cancer (World Health Organization) concludes that “shift work that involves circadian disruption is probably carcinogenic to humans” with an estimated risk level 2A, that is, close to full evidence (15). Thus, the prevention of circadian disruption, and/or the restoration of functional clocks, could constitute new objectives for therapeutics.

Chronotherapeutics aims at improving the tolerability and/or the efficacy of medications through the administration of treatments according to biological rhythms (8, 16). The adequate adjustment of treatment delivery to physiological rhythms and the restoration or the induction of these rhythms can improve therapeutic outcomes in cancer patients (8, 17).

Recent advances identify critical molecular events that rhythmically control drug metabolism and detoxification, cell cycle, molecular targets, DNA repair, apoptosis, and angiogenesis. The coordination of these processes along the 24-h period is ensured by the circadian timing system (CTS), whose hierarchical organization determines chronotherapeutic effects. Phase I to III clinical trials validate the relevance of circadian timing of cancer treatments. Moreover, translational studies identify potential key determinants to optimally shape circadian drug delivery patterns in a given patient. Data-based computational models are providing novel insights into the interactions between circadian clocks, cell cycle, and anticancer drug pharmacology. They now reveal several critical dynamic events for the success of cancer chronotherapeutics through the design of patient-tailored chronomodulated delivery of anticancer medications.

THE CIRCADIAN TIMING SYSTEM

Overall Organization

The CTS coordinates physiology and cellular functions over a 24-h period. Environmental synchronizers such as the alternation of days and nights, socio-professional routines, and meal times

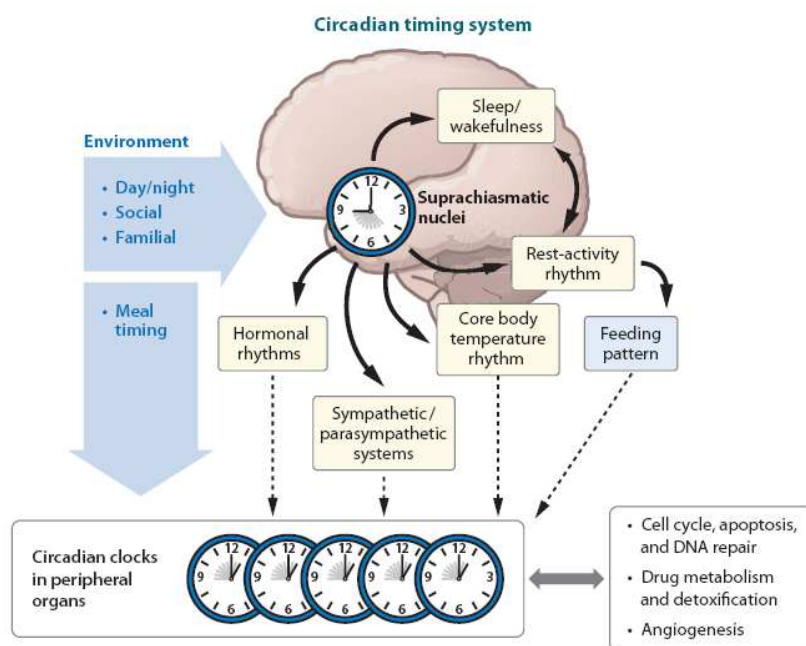


Figure 1

Schematic representation of the CTS. The CTS is composed of (a) a hypothalamic pacemaker, the suprachiasmatic nuclei SCN, (b) an array of SCN-generated circadian physiology outputs, and (c) molecular clocks in the cells of all peripheral tissues. Molecular clocks rhythmically control xenobiotic metabolism and detoxification, cell cycle, apoptosis, DNA repair, and angiogenesis over a 24-h period. The CTS is synchronized with time cues provided by light-dark cycles and other environmental factors. Circadian physiology outputs can also serve as CTS biomarkers.

entrain and calibrate at precisely 24 h, the period of the CTS (**Figure 1**). Endogenous circadian rhythms with periods differing from precisely 24 h characterize all aspects of mammalian physiology (10, 18, 19). In human beings synchronized with usual light-dark, socio-professional, and feeding synchronizers, motor activity is high at daytime and low at night, body temperature reaches a maximum in the early evening, cortisol secretion by the adrenal gland rapidly rises from a nadir near 2:00 a.m. to a maximum near 8:00 a.m., and melatonin secretion by the pineal gland mostly occurs at night, with a maximum near 2:00 a.m. (18, 19). This circadian physiology is generated or controlled by a central pacemaker, the suprachiasmatic nuclei (SCN), in the hypothalamus. The circadian period of the SCN neurons is calibrated to 24 h through the perception of synchronization signals, namely light and darkness via the retino-hypothalamic tract using glutamate and pituitary-adenylate-cyclase-activating peptide (PACAP) as neuromediators and other brain areas via neuropeptide Y fibers (18). The SCN generates circadian physiology through diffusible signals, including transforming growth factor α , epidermal growth factor, prokineticin-2 (PK-2), cardiotrophin-like cytokine, and neuroanatomic sympathetic and parasympathetic pathways (20–22). Circadian physiology and other signals directly or indirectly emanating from the SCN coordinate molecular clocks in each cell (18, 23). In turn, the molecular clock rhythmically

Clock: circadian locomotor output cycles kaput
Bmal: brain and muscle aryl hydrocarbon receptor nuclear translocator
Per: period (gene or protein)
Cry: cryptochrome
DBP: albumin D-binding protein
TEF: thyrotroph embryonic factor
HLF: hepatic leukemia factor

controls many cellular functions that are relevant for cancer treatment including drug metabolism and detoxification as well as cellular proliferation, DNA damage sensing and repair, apoptosis, and angiogenesis (24).

The periodic resetting of the circadian time structure by external 24-h cycles allows for the prediction of times of the peaks and troughs of circadian rhythms in rodents and in humans. This applies to the rhythms that regulate anticancer drug pharmacology and cellular proliferation (23, 24). Conversely, a lack of external synchronizers, that is, a defect in the perception of environmental time cues through blindness, for instance, or an alteration of the circadian physiology, molecular clock, or clock-controlled pathways, results in the deregulation of the circadian time structure (19, 25, 26). In turn, relevant 24-h rhythms become damped, ablated, or phase shifted, with an unpredictable timing of the peaks and troughs if the circadian period is lengthened, shortened, or shifted. In such cases, melatonin, glucocorticoids, or other chronobiotic agents can restore proper circadian coordination (26, 27).

Healthy human subjects can display different CTS phasing, despite exposure to the same environmental synchronizers. Such distinct chronotypes are defined with questionnaires on living habits, which reflect distinct timing of circadian behavior, physiology, and clock gene expression patterns (28).

Circadian Clock Mechanisms

A dozen specific clock genes constitute the core of the molecular clock in mammals (Figure 2). These genes are involved in transcriptional and posttranscriptional activation and inhibition regulatory loops that result in the generation of the circadian oscillation in individual mammalian cells. In particular, the CLOCK-BMAL1 or NPAS2-BMAL1 protein dimers play a key role in the molecular clock through the activation of the transcription of the clock genes *Per* and *Cry* (23, 27). The functionality of the molecular clock in peripheral tissues including malignant tumors can be estimated through the relative phase relations of circadian expression patterns of three core clock genes whose transcription is regulated by one another: *Rev-erba* downregulates *Bmal1*, *Bmal1* upregulates *Rev-erba* and *Per2*, and *Per2* downregulates *Rev-erba* and its own transcription (23, 24).

The CLOCK-BMAL1 transactivation complex also rhythmically controls the mRNA transcription of proline-acidic amino acid-rich basic leucine zipper (PAR bZip) transcription factors, including albumin D-binding protein (DBP), thyrotroph embryonic factor (TEF), and hepatic leukemia factor (HLF) (29). These transcription factors regulate most pathways that handle xenobiotic metabolism and detoxification in liver, intestine, and kidney through the rhythmic control of C-androstane receptor, P450 oxydo-reductases, and 5-amino- δ -levulinic acid synthetase (*Alas1*) (23, 29). Furthermore, posttranslational modifications regulate ticking of the molecular clock (30).

The CLOCK-BMAL1 dimer also gates cell cycle phase transitions through the repression of *c-Myc* and *p21*, two important players in cellular proliferation and apoptosis, the activation of *p53*, a proapoptotic gene, and that of *Wee1*, whose protein gates transition from G₂ to mitosis (24, 31, 32). Circadian clocks further regulate apoptosis through the rhythmic expressions of antiapoptotic BCL-2 protein and proapoptotic BAX protein (33); DNA damage sensing through molecular interactions of ataxia telangiectasia mutated (ATM) or ATM and rad3-related interacting protein (ATRIP) with clock proteins PERs, CRYs, and TIM (32, 34); and DNA repair through rhythmic activities or levels of O₆-methylguanine DNA methyltransferase, a protein that excises lethal DNA alkylated lesions produced by nitrosoureas (35), as well as *Tip60*, *Xpa*, and possibly *Erc1*, which repair platinum-induced DNA adducts (36–38).

The intrinsic sustainability of molecular clocks has been shown in synchronized cell cultures. Thus, cell lines are potential models for in vitro studies of circadian clocks and clock-controlled

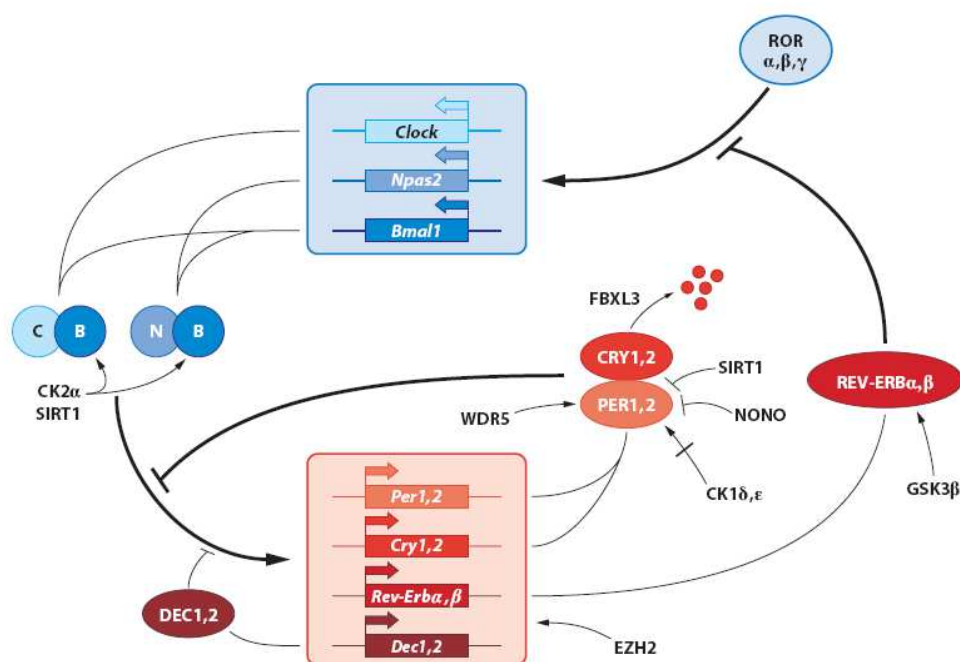


Figure 2

Simplified hypothetical mammalian circadian clock. The molecular oscillator is thought to be based on molecular feedback loops within a positive limb (CLOCK, NPAS2, BMAL1) and a negative limb (PER and CRY) that are interconnected via the nuclear orphan receptor REV-ERB α . The transcription of *Per* and *Cry* genes is activated by heterodimers between BMAL1 (B) and either of the two related proteins CLOCK (C) or NPAS2 (N). The polycomb protein EZH2 as well as casein kinase 2 (CK2) and silencing information regulator SIRT1 interact with these heterodimers and thereby facilitate their action. The accumulation and activity of PER and CRY proteins are also influenced by phosphorylation by protein kinases (CK1 δ , ϵ), by ubiquitination via a complex containing the F-box protein FBXL3 (specific for CRYs), by the histone methyl-transferase-binding protein WDR5, and by NONO, an RNA- and DNA-binding protein. DEC1 and DEC2 compete with BMAL1-CLOCK/NPAS2 heterodimers for E-box binding and thereby reduce E-box-mediated transactivation. An accessory feedback loop, employing the nuclear orphan receptors ROR α , ROR β , and ROR γ as activators, and REV-ERB α and REV-ERB β as repressors, regulates the circadian transcription of *Bmal1*. (Adapted from U. Schibler, with permission.)

pathways (39). Two-hour exposure of cultured cells to 50% horse serum, dexamethasone, or other compounds synchronizes the circadian clocks in cultured cells whose internal timing is otherwise drifting at a different pace (39, 40). Circadian transcription has been demonstrated for at least three full periods in synchronized cultures of cell lines and ex vivo cellular preparation or tissue explants from rodents or humans, including SCN, liver, lung, kidney, intestine, and adipose tissue (41–43). The use of a PERIOD2-LUCIFERASE fusion protein as a real-time reporter of circadian dynamics demonstrates that peripheral tissues from mice self-sustain circadian oscillations for >20 cycles in isolation, with tissue-specific differences in circadian period and phase (44). Repeat serum shocks at 3-day intervals or 24-h cycles in external temperature avoid the desynchronization of in vitro transcription circadian rhythms (39, 45). The properties of synchronized cell cultures thus support their recent use as potential models for cellular chronopharmacology.

ZT: Zeitgeber time (equivalent to hours after light onset)

THE EXPERIMENTAL CHRONOPHARMACOLOGY OF ANTICANCER AGENTS

The Relevance of Circadian Timing for Treatment Tolerability

Circadian timing largely modifies the extent of toxicity of 40 anticancer drugs, including cytostatics, cytokines, and targeted biological agents, in mice or rats (**Table 1**). A potentially lethal dose of any of these agents results in 2-fold to more than 10-fold changes in the incidence of toxic deaths and/or maximum body weight loss as a function of circadian timing of drug administration. Such large differences occur irrespective of delivery route—oral, intravenous, intraperitoneal, or intra-arterial—or the number of daily or weekly administrations (23). The methodology used to demonstrate the 24-h changes in anticancer drug tolerability involves the synchronization of nocturnally active mice or rats with an alternation of 12 h of light and 12 h of darkness (LD12:12). The same drug dose is administered to different groups of rats or mice, with each group corresponding to a different circadian stage, also called Zeitgeber time (ZT). Usually, six circadian stages, occurring 4 h apart, are tested. Time usually is expressed in ZT hours or in hours after light onset. Dedicated chronobiologic animal facilities allow setup light onset at the desired time for different groups of animals located on different isolated shelves, so that different circadian stages are tested at the most convenient times (46). **Figure 3** depicts the times of least toxicity and benefit from optimal circadian timing, referring to external LD synchronizer and internal average body temperature rhythm for 16 anticancer drugs in male B6D2F1 mice (female C57BL/6 × male DBA2) in studies performed at our laboratory (47–63). The optimal circadian timings are staggered along the 24-h period and cannot be predicted thus far by the knowledge of pharmacologic class or that of main target organs for toxicity. Circadian rhythms in the tolerability of anticancer drugs persist in rodents kept in constant darkness or in constant light, which demonstrates their endogeneity (64).

Even when combined, chemotherapeutic agents display the least toxicity near their respective times of best tolerability as single agents, as shown for doxorubicin-cisplatin in Lou rats, irinotecan-oxaliplatin or gemcitabine-cisplatin in B6D2F1 mice, and docetaxel-doxorubicin in C3H/He mice (56, 65–67). These findings support the persistence of the circadian control of anticancer drug determinants after exposure to the first anticancer agent, at least when the latter is given near the time of best tolerability.

Circadian Control of Metabolism, Detoxification, and Pharmacokinetics

Most anticancer agents with circadian tolerability undergo oxidation, reduction, or hydrolysis under phase I metabolism, mainly in the liver and, to a lesser extent, in the intestine (1, 2). The CTS controls both phase I metabolism and phase II drug detoxification and elimination through redundant processes involving rhythmic physiology and circadian clock signaling (**Figure 4**) (23, 29, 68, 69).

The activity of most microsomal oxidases are highest by severalfold during the dark (activity) span and lowest during the light (rest) span in the liver of rats and mice (29, 68, 70). Twenty-four-hour rhythms further characterize the activities of several CYP isoenzymes (23, 71). Two- to eight-fold circadian changes in mRNA expression are found for cytochrome P-450 oxidoreductase in liver and intestine, with a maximum at ZT12; for *Cyp2b10* (testosterone 16- α -hydroxylase), with a maximum at ZT16 in liver and intestine; and for *Cyp2c50*, with a maximum at ZT20 in mouse liver (29). However, *Cyp3a13* may escape from clock regulation (72).

The circadian rhythm in *Cyp3a* likely contributes to the chronotolerance pattern of seliciclib, docetaxel, irinotecan, mitoxantrone, and vinorelbine, which undergo oxidative metabolism. On

Table 1 Anticancer drugs with documented relevance of circadian timing for tolerability, pharmacokinetics, and/or antitumor efficacy in laboratory rodents

Pharmacologic class	Drug	Endpoint modified by circadian timing [reference(s)]		
		Tolerance	Pharmacokinetics	Efficacy
Antimetabolite	D-actinomycin	(219)		
	Methotrexate	(220, 221)	(99, 221)	(99)
	5-fluorouracil	(88, 222, 223)	(86, 224)	(88, 225)
	Floxuridine	(226, 227)		(226, 228)
	Arabinofuranosylcytosine	(229)		(229, 230)
	Gemcitabine	(56)		(56)
Top 1 inhibitor	L-alanosine	(55)		(55)
	Irinotecan	(48, 77, 82)	(77, 82)	(66)
	Topotecan	(95)		(95)
Top 2 inhibitor	9-aminocamptothecin	(231)		(231)
	Mitoxantrone	(54)	(54)	
DNA intercalator	Etoposide	(52)		
	Daunorubicin	(232)		
Mitotic inhibitor	Doxorubicin	(64, 67, 233, 234)	(234)	(65, 67, 235, 236)
	Doxorubicin-liposomes	(237)		(237)
	Theprubicin	(46, 53, 238)		(239)
	Epirubicin	(240)		
Alkylator	Vincristine	(241)		
	Vinblastine	(242)		
	Vinorelbine	(63, 182)		(182)
	Docetaxel	(58, 67)		(58, 67)
Nitrosourea	Cyclophosphamide	(72, 91)	(72)	(230, 236, 243–245)
	Ifosfamide	(246)		
	Melphalan	(235, 247)		(235)
	Peptichemo	(51)		
	Mitomycin-C	(62)		
	Cisplatin	(47, 59, 212)	(47, 248)	(56, 65, 249)
	Carboplatin	(47, 60, 103)	(47, 59)	
	Oxaliplatin	(59, 61)	(59, 61, 87, 250)	(66)
	B-85-0040	(251)		(251)
Cytokines	Nedaplatin	(252)	(252)	
	Cysteamine	(57)		(57)
	rHu-interferon α	(253)		(253)
	rMu-interferon γ	(253)		(253)
	Interferon β	(98)	(98)	(98)
CDKI	Tumor necrosis factor α	(254)		(255)
	Interleukin-2	(50)		(256)
Cox-2 inhibitor	Selaciclib	(49)	(49)	(94)
VEGF inhibitor	Celecoxib	(96)		(96)
TNP-470	TNP-470	(97)	(97)	(97)
	SU 1498			(100)
	BB2516			(100)

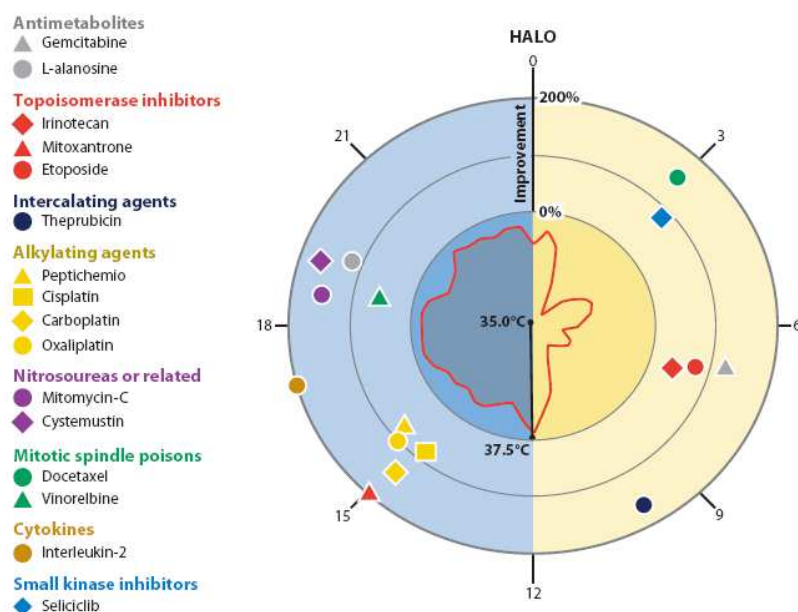


Figure 3

Relevance of circadian timing for the tolerability of anticancer drugs. Circadian timing associated with best tolerability, in ZT (or hours after light onset, ranging from 0 to 24) and relative magnitude of survival benefit from optimal to worst timing, ranging from 0 to 200%. The diagram illustrates chronotolerance for 16 anticancer drugs studied in our laboratory in male B6D2F1 mice, synchronized with LD12:12. The average circadian rhythm in body temperature is shown in the internal circle and provides a CTS biomarker, an endogenous reference for optimal drug timing (see Table 1 for corresponding references).

the other hand, nonrhythmic *Cyp3a13*, rhythmic *Cyp2b10*, and possibly *Cyp2c29* participate in the circadian tolerability of cyclophosphamide (29, 72). In contrast, 5-FU and gemcitabine, whose toxicity also depends upon circadian timing, undergo rapid liver catabolism through dihydropyrimidine dehydrogenase (DPYD) and cytidine deaminase activities, respectively (73–75).

The carboxylesterases *Ces1* and *Ces2* are rhythmically controlled both by the circadian clock and by clock-controlled *Dbp*, *Tef*, and *Hlf* in the liver and gastrointestinal tract (29, 76). *Ces1* and *Ces2* circadian expression can account for the increased biotransformation of irinotecan into SN-38 during the light (rest) span of male ICR mice (77). *Dpyd* mRNA expression and activity display significant rhythms in the liver of male B6D2F1 mice, with an ~15-h time lag between their peaks. Peak DPYD activity occurs near the middle of the light span, when the animals rest (78). All the enzymatic activities that generate the cytotoxic forms of 5-FU, such as orotate phosphoribosyltransferase, uridine phosphorylase, and deoxythymidine kinase, are highest during the dark (activity) span of rats or mice, when 5-FU is most toxic to healthy tissues (23).

Rhythmic phase II detoxification by reduced glutathione (GSH) is a critical determinant of the toxicities of platinum complexes and other cytostatics. Liver and jejunum GSH contents are approximately threefold higher in the second half of the dark span in mice and rats compared with mid-light, and the pharmacologic suppression of GSH synthesis with buthionine

GSH: reduced glutathione

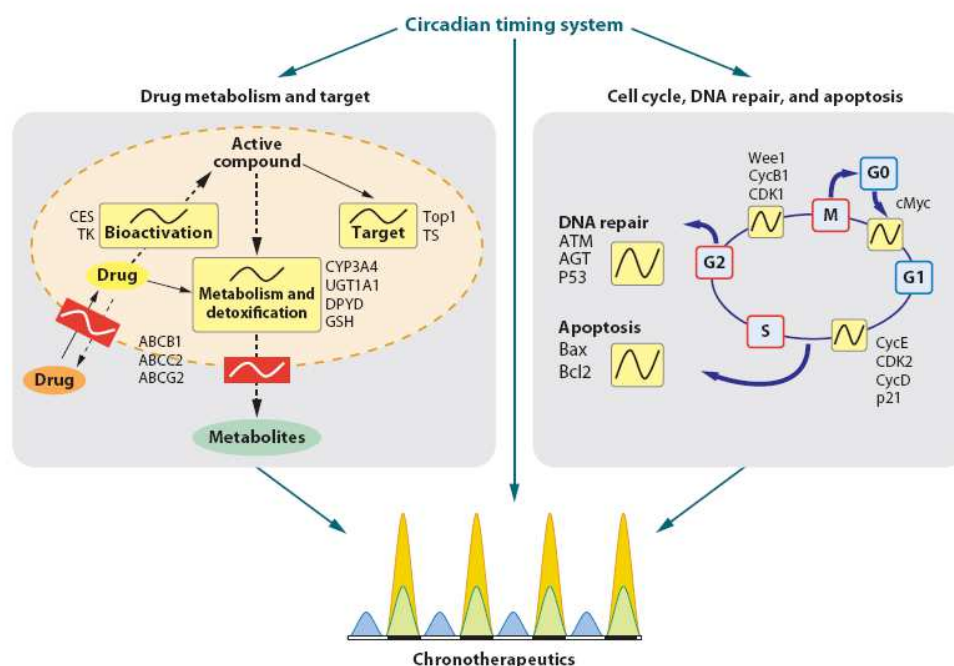


Figure 4

Main cellular determinants of cancer chronotherapeutics. The CTS (*top*) determines the optimal circadian timing of anticancer medications (*bottom*). The CTS controls drug transport, bioactivation, detoxification, metabolism, targets, and elimination, which account for the chronopharmacology of anticancer agents at cellular, tissue, and whole organism levels (*left*). The CTS also regulates several cell-cycle-related events that gate G1/S or G2/M transitions, as well as DNA repair and apoptosis, which account for the chronopharmacodynamics of anticancer drugs (*right*). The relations between chronopharmacokinetics and chronopharmacodynamics help construct optimal chronomodulated drug delivery schedules, with proper parameters.

sulfoximide profoundly alters the chronotolerance pattern of cisplatin and oxaliplatin in mice (79, 80).

UGT1A catalyzes the detoxification of seliciclib, irinotecan, and SN-38. Highest UGT activity is reported during the dark (activity) span of rats (70). However, mean and circadian expression of *Ugt1a1* differ according to species, strain, and gender, with relevant consequences for irinotecan chronotolerance (81, 82). Circadian clocks control the transcription of ATP-binding cassette family members, including *Abcb1a* and *Abcb1b* (*Mdr1*), *Abcc2* (*Mrp2*), and *Abcb4* (*Mdr2*) in mouse liver and intestine (29, 69, 83–85). The *Abcb1a* and *Abcb1b* mRNA rhythms translate into a 30% increase of permeability glycoprotein (*P-gp*) activity at ZT17–19 compared with ZT5–7 in rat jejunum and ileum (B. Lemmer & A. Okyar, unpublished data).

The multiple circadian controls of drug absorption, distribution, metabolism, and elimination (ADME) account for dosing time dependencies in the pharmacokinetics of 17 anticancer drugs of all classes in mice, rats, and even pigs (Table 1). Even the continuous delivery of 5-FU by an implanted pellet results in circadian changes in plasma drug concentrations, with high values at daytime, during the rest span of the mice (86). Circadian timing mostly affects the initial distribution phase (C_{max} , $t_{1/2\alpha}$, V_{di}), the area under the concentration \times time curve (AUC), and the plasma

P-gp: permeability glycoprotein

TS: thymidilate synthetase

Top: topoisomerase

clearance of anticancer agents. High circadian drug exposure of healthy tissues, based on plasma PK analysis, is related to the high circadian toxicity of methotrexate, mitoxantrone, interferon- α , and the antiangiogenic agent TNP-470 in mice (**Table 1**). However, no consistent relationships are found between blood chronopharmacokinetics and chronotolerance for irinotecan, cyclophosphamide, cisplatin, carboplatin, oxaliplatin, interferon β , or seliciclib. For instance, highest Vdi and elimination are observed in mice dosed with carboplatin at ZT8 and with oxaliplatin at ZT16, despite both drugs being least toxic at ZT16 (87). Highest platinum content is found in 12/18 tissues 24 h after a single dose of oxaliplatin at ZT8, when this drug is most toxic (61). However, no consistent relationship is found in the tissues of mice treated at ZT 24, when the toxicity of oxaliplatin is intermediate, or at ZT16, when it is least toxic (61). The plasma and liver pharmacokinetics of orally given seliciclib differ significantly according to circadian timing. Seliciclib AUC is 25% greater at ZT3 than at ZT19 in plasma, and 80% less at ZT3 than at ZT19 in liver, when the drug produces the fewest liver alterations (49). Taken together, the results both emphasize and qualify the relevance of chronopharmacokinetics as mechanisms of chronotolerance for anticancer medications.

Circadian Control of Cell Cycle, DNA Repair, Apoptosis, and Molecular Targets

Cell cycle events are coordinated along the 24-h period in healthy bone marrow, gut, and skin, three frequent targets for the toxicity of cancer treatments (24). Proportions of S- and G2/M-phase cells increase by $\sim 50\%$ in the second half of darkness, whereas G0-G1 cells predominate during light in the total bone marrow of male B6D2F1 mice (33, 58). In this tissue, BCL2 protein expression triples over the 24-h period, with a maximum at early light. An opposite pattern characterizes proapoptotic BAX, with a fivefold 24-h change and a peak at ZT15 (33). The temporary arrest of cycling cells in G0-G1, the high BCL2, and low BAX expressions during the light span when mice rest help explain the best circadian timing for the tolerability of 5-FU, gemcitabine, irinotecan, and docetaxel in male B6D2F1 mice (48, 56, 58, 67, 73). However, the circadian control of drug metabolism and detoxification also profoundly modifies the cellular exposure to these medications, whose molecular targets are usually clock regulated (77, 88). For instance, the increased detoxification of 5-FU during the light span results from the circadian peak in DPYD activity in liver and other healthy cells (73, 78). It thus adds up to the reduced proportions of S-phase cells in bone marrow, gut, and skin, as a mechanism for improved circadian tolerability (73). Both transcription and activity of thymidilate synthetase (TS), which provide the unique de novo source of thymidilate, are linked to early S-phase in proliferating tissues (89). Consistently, bone marrow TS activity peaks near mid-dark in coincidence with the greatest hematologic toxicity of 5-FU in female CD2F1 mice (88).

Whereas circadian Phase I and II metabolism partly determines irinotecan pharmacology over the 24-h period, topoisomerase 1 (Top1), the main protein target of this drug is mostly at work during late S-phase. Thus, the circadian gating of the cell cycle and possibly the direct control of Top1 by the molecular clock also contribute to the better hematologic tolerability of irinotecan during the rest span of male ICR mice (77, 90).

The Role of Molecular Clocks and Clock-Controlled Pathways in Chronotolerance

The CLOCK-BMAL1 transactivation complex represses cyclophosphamide toxicity mechanisms and partly determines the chronotolerance pattern of this drug. Cyclophosphamide was best tolerated at ZT10-ZT14 in two studies carried out ~ 30 years apart in female wt C57Bl6 mice (72, 91).

Cyclophosphamide tolerability is worse in *Clock^{tm/m}* and *Bmal1^{-/-}* mice, whereas it is improved in *Cry1^{-/-}Cry2^{-/-}* mice (72). In these three strains with a genetically disrupted molecular clock, chronotolerance patterns of cyclophosphamide are blunted (72). Circadian pharmacokinetics result in greatest formation of 4-OH-cyclophosphamide and dechloroethylcyclophosphamide in wt mice dosed at ZT2, when the drug is most toxic. *Clock^{tm/m}* increases formation of the bioactive 4-OH-cyclophosphamide and profoundly modifies cyclophosphamide metabolism (72). The tolerability of cyclophosphamide, mitoxantrone, vincristine, and methotrexate is best near ZT12, when *Dbp*, *Tef*, and *Hlf* expressions are high (29, 92). Whereas vincristine and methotrexate show no significant differences in toxicity between wt and triple *Dbp*, *Tef*, *Hlf* knockout mice; both mitoxantrone and cyclophosphamide are much more harmful in PAR bZip-deficient as compared with PAR bZip-proficient animals (29). Clock-controlled PAR bZip transcription factors play a critical role in the detoxification of anticancer drugs whose metabolisms involve carboxylesterases (CES), sulfotransferase, UGT1A, glutathione S-transferase, and ABC transporters such as P-gp and breast cancer resistance protein (BCRP) that are responsible for the intestinal and biliary secretions of several anticancer drugs (29).

ABC transporter:
ATP-binding cassette
transporter

GOS: Glasgow
osteosarcoma

The Relevance of Drug Timing for Treatment Efficacy

Circadian timing also critically affects antitumor efficacy of 28 anticancer medications, including cytostatics, antiangiogenic agents, and cell cycle or Cox2 inhibitors in rodents with various kinds of malignancies (Table 1). The demonstration of chronoefficacy is based on the administration of a single agent for several days or weeks and/or its combination with up to four other drugs at stipulated circadian times (93). Appropriately circadian-timed and dosed chemotherapy with one or several drugs at least halves tumor growth rate and/or significantly increases life span in tumor-bearing mice (56, 66, 94). Circadian timing also largely modifies the efficacy of anticancer agents against human cancer cells from breast (MCF-7, ZR-75-30, and MDA-MB-468) or colon (HCT116) transplanted into nude mice (95, 96).

Strikingly, the circadian pattern in chronoefficacy usually coincides with that in chronotolerance (Table 1). This is true for cytostatics, interferons, antiangiogenic agents, and cell cycle inhibitors, as well as for combination chemotherapy, such as irinotecan-oxaliplatin, gemcitabine-cisplatin, and docetaxel-doxorubicin, three widely used clinical regimens (56, 66, 67, 97, 98). Experimental chronotherapeutics thus strongly supports circadian timing as a relevant method for improving anticancer treatments.

The chronoefficacy of anticancer medications can partly result from circadian changes in tumor drug uptake, as shown for methotrexate in sarcoma-bearing rats, interferon β in mice with B16 melanoma, and seliciclib in mice with Glasgow osteosarcoma (GOS) (94, 98, 99) (M. Hassan, E. Filipski, & F. Lévi, unpublished data). Chronoefficacy can also stem from the circadian control of drug pharmacodynamics in tumors, as shown for cell cycle phase distribution, related protein targets, such as TS for 5-FU and Top1 for irinotecan and receptors, such as interferon- α/β receptors (88, 90, 98). Vascular endothelial growth factor is also produced rhythmically in slow-growing mouse sarcoma-180 with a maximum near ZT2, when the antitumor efficacy of three antiangiogenic agents doubles compared with an administration at ZT14 (100).

However, circadian disruption frequently adds to cell cycle disruption as a hallmark of cancer, at least in rapidly growing malignancies and at an advanced stage of tumor evolution (14, 24, 32, 34, 93, 94). Clock gene transcription is no longer circadian in advanced GOS or pancreatic adenocarcinoma P03 (14, 94) (X. M. Li, F. Delaunay, S. Dulong, B. Claustrat, S. Zampera, et al., submitted manuscript). No circadian organization is found for S-phase cells in GOS or mammary carcinoma MA13C, for BCL2 protein expression in MA13C, or for GSH content in P03 (14, 33,

93, 94). Nevertheless, chronoefficacy remains robust in these experimental tumors (56, 58, 93, 94), possibly because (a) the CTS of the host determines the chronoefficacy of anticancer medications, and/or (b) an adequate resetting of tumor circadian clocks by anticancer medications critically contributes to their efficacy.

The Circadian Timing System as a Target for Anticancer Treatments

Treatment-induced circadian disruption. Biomarkers of CTS coordination such as rest-activity and core body temperature can be severely disrupted by anticancer agents of any pharmacologic class (Table 2). This is also the case for rhythms in urinary excretion, blood cell counts, and other circadian biomarkers of chemotherapy toxicities. Anticancer agents also impair molecular circadian clocks in the SCN, liver, adrenals, or other peripheral organs of mice and in cell cultures (49, 101). Table 2 shows the disruption of host circadian rhythms for 12 anticancer medications in experimental models.

The extent of alterations and the recovery dynamics of rest-activity and body temperature rhythms vary as a function of both dose and circadian timing, as shown for vinorelbine and gemcitabine (74, 102). For instance, a single therapeutic high dose of gemcitabine mildly alters both SCN biomarkers if the drug is given at ZT11, but it markedly suppresses them if it is administered at ZT23, when it is most hematotoxic (Figure 5) (56, 74). Circadian timing determines the extent and duration of SCN disruption produced by a single therapeutic dose of irinotecan, oxaliplatin, vinorelbine, interferon- α , or seliciclib (74, 101, 102) (Table 2). Conversely, treatment at the circadian time associated with fewest toxicities best spares the CTS, irrespective of the underlying toxicity mechanisms or target tissues. Similar findings characterize circadian biomarkers of tissue toxicity. Thus, blood cell count rhythms are maintained or suppressed in mice dosed with theprubicin and carboplatin, near their respective best or worst circadian timing (46, 53, 103).

Circadian disruption also affects the molecular clocks in the central pacemaker and in peripheral tissues. The circadian patterns in mRNA expression of *Per1*, *Per2*, *Per3*, and *Bmal1* in the SCN are ablated in mice receiving interferon- α at ZT12 and maintained in those treated at ZT0 (101). Mice on interferon- α at ZT0 also maintain near normal *Per1* mRNA rhythm in liver and in adrenals. Conversely, *Per1* expression rhythm is damped and advanced by ~4 h in the mice treated at ZT12 (101). Inappropriately timed anticancer agents can modify circadian clock amplitude and phase in peripheral organs and prevent the predictability of internal circadian timing (46, 74, 101–104). Constant-rate infusion of 5-FU with an osmotic minipump attenuates the circadian rhythms in *Per1* and *Per2* mRNA in both SCN and liver of mice (104). Seliciclib, a cyclin-dependent kinase (CDK) inhibitor, either profoundly disrupts *Rev-erb α* , *Per2*, and *Bmal1* transcription rhythms at ZT3 or only dampens their patterns at ZT19 in mouse liver (Figure 5) (49). The circadian disruption of the liver clock at ZT3 is selectively associated with liver toxicity (49). Dose-dependent disruption of circadian clocks also results from in vitro exposure of synchronized NIH3T3 fibroblasts or Period2-Luciferase expressing C6 glioma cells to 5-FU (104).

Circadian induction by anticancer agents. Spontaneous or imposed rhythmic patterns in corticosterone, body temperature, or feeding entrain relevant cell cycle and pharmacology determinants over the 24-h period in premalignant tissues or in tumors and slow down cancer processes (14, 90) (X. M. Li, F. Delaunay, S. Dulong, B. Claustrat, S. Zampera, et al., submitted manuscript). DNA repair elicited by γ -radiations and possibly also by anticancer drugs resets free-running host circadian clocks via ATM-mediated damage signaling (105). However, tumors frequently escape from circadian coordination. Thus, no circadian expression pattern is found for *Per2*, *Bmal1*, and

Table 2 Effects of anticancer drugs on the circadian timing system in experimental models

Class of agent	Name	Test system	Disrupted rhythm	Role of circadian timing	Reference
Antimetabolite	5-fluorouracil	Mouse (male ICR)	Locomotor activity <i>Per 1, 2</i> in liver & SCN	NA	(104)
		Mouse NIH3T3 (culture)	<i>Per 1, 2</i>	NA	
	L-alanosine	Mouse (male B6D2F1)	Rest-activity Body temperature	NA	(55)
	Gemcitabine	Mouse (male B6D2F1)	Rest-activity Body temperature	Yes	(74)
Top1 inhibitor	Irinotecan	Mouse (male B6D2F1)	Rest-activity Body temperature	Yes	C. Ahowesso & F. Lévi (unpublished data)
Intercalators	Theprubicin	Mouse (male B6D2F1)	Blood cell rhythms	Yes	(46)
Alkylators	Cisplatin	Rat (female F344)	Body temperature Urinary rhythms	Yes	(257)
	Oxaliplatin	Mouse (male B6D2F1)	Rest-activity Body temperature	Yes	E. Filipinski & F. Lévi (unpublished data)
	Carboplatin	Mouse (male ICR)	Blood cell rhythms	Yes	(103)
Mitosis inhibitor	Vinorelbine	Mouse (male B6D2F1)	Rest-activity Body temperature	Yes	(102)
Radiation	γ -radiation	Mouse (male C57BL/6J)	Locomotor activity	Yes	(105)
		Rat fibroblasts	<i>Per 1, Per 2, Clock, Bmal1</i>	Yes	
Cell cycle inhibitor	Seliciclib	Mouse (male B6D2F1)	Rest-activity Body temperature <i>Rev-erba, Per 2, Bmal1</i> in liver	Yes	E. Filipinski & F. Lévi (unpublished data) (49)
Cytokine	Interferon- α	Mouse (male ICR)	Locomotor activity Body temperature <i>Per 1, 2, 3; Clock; Bmal1</i> in SCN, liver, adrenals	Yes	(258) (101)
		HepG2 (culture)	<i>Clock & Bmal1</i> mRNA & protein	NA	(259)

Rev-erba in advanced GOS or pancreatic adenocarcinoma P03 (14, 94) (X.M. Li, F. Delaunay, S. Dulong, B. Claustrat, S. Zampera, et al., submitted manuscript). An induction of near normal circadian patterns in clock gene transcription is produced in GOS, with five daily oral administrations of seliciclib at ZT3, but not at ZT19. Tumor clock induction by seliciclib at ZT3 nearly doubles the antitumor efficacy of seliciclib as compared with ZT19 treatment. Mechanisms involve the transient inhibition of casein kinase I δ/ϵ , an enzyme that regulates the intrinsic period of the circadian clock itself and translates into differential expressions of clock-controlled genes *c-Myc* and *Wee1* (94).

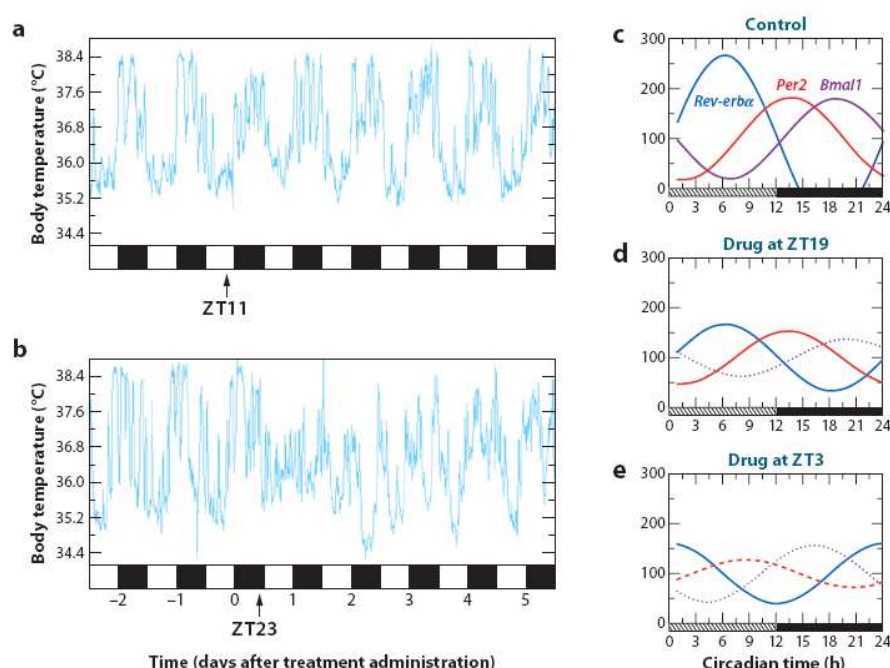


Figure 5

Circadian disruption resulting from anticancer medications. (Left) Effect of a single dose of gemcitabine (400 mg/kg iv) on the circadian rhythm in intraperitoneal temperature recorded via telemetry. The drug administration (vertical arrow) is at (a) ZT11 or (b) ZT23 (disruption), when it also achieves best or worst hematologic tolerability, respectively. Adapted with permission from Reference 74. (Right) Effects of five daily doses of seliciclib (300 mg/kg/d po) on the liver molecular clock as estimated with transcriptional rhythms in *Rev-erba* (blue line), *Per2* (red line), and *Bmal1* (purple line). Solid lines correspond to statistically validated 24-h rhythms. (c) Control liver clock. Drug administration is at (d) ZT19 or (e) ZT23 (disruption), when it achieves best or worst hepatic tolerability, respectively. Adapted with permission from Reference 49. Both studies involve male B6D2F1 mice synchronized by LD12:12 (alternation of open and dark boxes).

Implications for cancer treatments. Most anticancer drugs that disrupt SCN biomarkers are moderately taken up in brain tissue, yet no specific information about SCN drug uptake exists (106, 107). Anticancer therapy can induce the release of several cytokines or growth factors that can also modify the CTS (108, 109). Transforming growth factor α , epidermal growth factor, CLC, and PK-2 suppress SCN biomarkers following their infusion in the third ventricle of mice or hamsters (20–22). These peptides can penetrate the brain from the systemic circulation (110–112). IL-6 induces *bPer1* in HU-H7 hepatoma cells (113). Tumor necrosis factor- α (TNF- α) disrupts circadian clocks and *Dbp*, *Tef*, and *Hlf* in the liver and in the SCN of mice, as well as *Per1*, *Per2*, and *Per3* circadian expression in cultured fibroblasts (114). Tumor necrosis factor- α inhibits CLOCK-BMAL1-induced activation of E-box regulatory-element-dependent clock gene promoters (114). Thus, the release of cytokines during toxic processes can disrupt host circadian clocks. In turn, circadian disruption accelerates malignant growth, as shown in mice with *Per2* suppression, SCN ablation, or chronic jet lag exposure (9, 10, 115). Direct effects on the clock

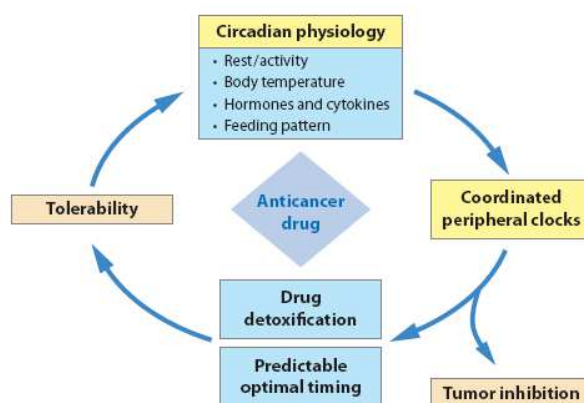


Figure 6

Scheme integrating the CTS in the therapeutic objectives of anticancer treatments. The adequate circadian timing of properly dosed anticancer medications ensures good treatment tolerability. In turn, adequate circadian physiology is maintained, molecular clocks in target tissues remain synchronized, drug metabolism and detoxification occur smoothly, and the optimal circadian timing remains predictable for subsequent drug administrations. In addition, the proper functioning of the CTS synergizes anticancer drugs with regard to their antitumor efficacy. In contrast, the elicitation of severe toxicities, through improperly timed and/or dosed medications, disrupts circadian physiology, desynchronizes molecular clocks, alters drug metabolism and detoxification, impairs the predictability of optimal circadian timing, and alleviates CTS control on tumor progression.

mechanisms or signaling pathways also have to be considered, especially for noncytotoxic agents such as seliciclib (94, 116).

An uncoupling within the host CTS could further increase the susceptibility of the organism to anticancer drugs, through an alteration of the fine-tuned circadian coordination of detoxification pathways (29). Circadian disruption then impairs the dynamics of detoxification and retards the recovery from toxicity at tissue and central levels, in good agreement with experimental chronotolerance data. High systemic toxicity of anticancer drugs seems to correlate with circadian disruption, and circadian disruption accelerates cancer progression (9, 10, 115). Cancer chronotherapeutics could then aim for the minimization of host clock disruption to prevent toxicities and the induction of tumor clocks to inhibit cancer progression (Figure 6).

STANDARDIZED CLINICAL CANCER CHRONOTHERAPEUTICS

Experimental chronotherapeutics suggest that some anticancer treatments are expected to be best tolerated and most effective at odd hours in cancer patients. This qualification is handled by dedicated drug delivery technologies, which further allow the design of novel circadian chronomodulated schedules. In turn, these schedules undergo validation steps for pharmacokinetics, clinical tolerability, and efficacy.

Drug Delivery Technology for Cancer Chronotherapeutics

The concept and the industrial development of nonimplantable multichannel programmable pumps have fostered the clinical development of cancer chronotherapeutics. Multiple circadian

ChronoFLO:
chronomodulated
delivery of 5-FU,
leucovorin, and
oxaliplatin

infusional schedules are jointly administered to nonhospitalized patients, with minimal or no medical or nursing intervention. The advent of Intelliject™ with four 30-ml reservoirs enabled the development of the first combination schedule of 5-FU-leucovorin-oxaliplatin and led to the initial demonstration of the safety and efficacy of this three-drug chemotherapy given according to a circadian chronomodulated delivery schedule, several years before the registration of oxaliplatin (17).

The approval of Intelliject in the European Union and in North America allowed its use for the routine chemotherapy of cancer patients as well for the evaluation of standardized chronomodulated infusions of 5-FU-leucovorin-oxaliplatin (ChronoFLO) within international clinical trials. Mélodie™, a second generation of electronically engineered four-channel programmable pumps, represents considerable technological progress, through increased energy autonomy, flexible reservoir capacity, rapid programming of any delivery schedule, computer storage of treatment protocols and patient data, as well as actual drug delivery reports for each treatment course. The infusional pressure of this pump allows the administration of irinotecan-5-FU-oxaliplatin in a European trial of three-drug chronomodulated infusions into the hepatic artery (117) (OPTILIV07: EUDRACT number: 2007-004632-24, ClinicalTrials.gov, identifier: NCT00852228). Preprogrammed, single-use, and elastomeric pumps with multiple electronic valves (CIP™) represent a recent concept of versatile multichannel chronotherapeutic drug delivery in ambulatory settings (118). Albeit lighter, ready-to-use, and disposable, the CIP requires modifications of drug delivery profiles to be performed using dedicated technology systems before use. This European Union-approved system is currently used both in daily oncology practice and in multicenter clinical trials (M. Pirovano & C. Garufi, personal communication). Conventional chemotherapy protocols only consider drug doses, duration, and frequency of infusions. As a result, treatment times vary among and within patients. However, 85% of the cancer treatments are administered from 9:00 a.m. to 5:00 p.m., that is, over only a third of the day span (119). In contrast, circadian chronomodulated schedules stipulate the time courses and parameters of the delivery profile for each anticancer medication over the 24-h period to achieve the best therapeutic index. This includes times of onset and offset of infusion and variation of flow rate, ranging from constant to sinusoidal or gradually increasing or decreasing.

Orally formulated anticancer medications are also amenable to chronotherapeutic delivery. Oral fluoropyrimidines seem to be best tolerated with systemic drug exposure at night (120–122). However, optimal chronotherapeutic delivery at night when the patient is sleeping requires adaptive drug delivery technologies. Novel oral pulsatile drug delivery systems release active drug principles after a predetermined lag time following ingestion and have proven their clinical relevance for chronotherapeutics (123, 124). Such systems could critically improve the safety and efficacy of orally dosed anticancer medications through circadian optimization of drug exposure.

Spontaneous or Imposed Circadian Control of Anticancer Drug Pharmacokinetics

Circadian timing significantly influences the plasma and/or urinary pharmacokinetics of intravenously administered 5-fluorouracil, methotrexate, doxorubicin, epirubicin, and cisplatin in cancer patients (125–130). This is also the case for orally administered busulfan, 6-mercaptopurine, and tegafur/uracil (120, 131–134). Continuous intravenous infusion results in 24-h changes in plasma concentrations for 5-FU (over 1–14 days), doxorubicin (over 2–42 days), and or vindesine (over 4 days) despite respective half-lives of 20 min, several hours, and 24 h (73, 135–138). The highest average plasma concentrations of 5-FU are found between 2:00 a.m. and 6:00 a.m. despite constant-rate infusion of this drug in 9 of 11 studies involving a total of 270 patients (23). The

duration of infusion, as well as patient gender, genotype, lifestyle, disease stage, and other drugs given concurrently can modify the average circadian pattern. Thus, both the mean and circadian amplitude of plasma clearance are halved in women as compared with men on a 2-day constant-rate infusion of 5-FU (139).

Circadian pharmacokinetics of anticancer drugs is found in children and in adults and deserves exploration in elderly cancer patients (140). Interpatient variability may mask chronopharmacokinetics, as shown in individual studies for oral methotrexate or 6-mercaptopurine, intravenous carboplatin, and continuous 5-FU or etoposide infusions (141–144). However, circadian changes of up to fivefold frequently characterize drug exposure in individual cancer patients. The mechanisms that drive human circadian pharmacokinetics partly match those already discussed in experimental models. Yet relevant dynamic biomarkers of drug metabolism and detoxification are usually lacking in cancer patients, except for the urinary excretion pattern of 6- β -OH-cortisol, which has been proposed as a biomarker of human CYP3A activity (145).

Chronomodulated infusions not only optimize drug exposure parameters according to circadian timing of peak delivery but also reduce interpatient variability. This is illustrated in two studies involving 27 patients with metastatic colorectal cancer receiving infusional 5-FU-leucovorin-oxaliplatin for 4 or 5 days (73, 87, 135). Total AUC of 5-FU varied fivefold among patients on constant-rate infusion and less than 0.7-fold in those on chronoFLO, despite the same dose (per square meter) being infused (73, 135). The drastic reduction in PK variability requires both chronomodulated infusion and the assignment of peak flow rate at 4:00 a.m. A highly statistically significant increase in inter- and inpatient variability characterizes the C_{max} of 5-FU if the drug delivery rate peaks at 1:00 p.m. or 7:00 p.m. (73, 135). Patients with a large regular circadian variation in the 5-FU plasma concentrations and a C_{max} located at 4:00 a.m. display best tolerability (73, 87, 135). The estimated total and free platinum AUCs are significantly lowest and most variable in patients receiving chronomodulated oxaliplatin with peak delivery at 1:00 a.m., compared with those with peak infusion rate at 7:00 a.m. or 4:00 p.m. (87). Diffusion of oxaliplatin out of the plasma compartment is likely to be greatest in the late evening or early night hours, when peripheral vascular resistance, plasma proteins, and erythrocyte membrane microviscosity are lowest (23, 146). The patients receiving chronomodulated oxaliplatin with a peak at 4:00 p.m. also experience less diarrhea and less peripheral sensory neuropathy than those with peak delivery at 1:00 a.m. or 7:00 a.m. (87). The relations between the circadian control of 5-FU and oxaliplatin pharmacokinetics and treatment tolerability have been confirmed in subsequent large clinical trials (147–149).

The relation between pharmacokinetics and toxicity has also been investigated in a randomized study involving 31 cancer patients receiving irinotecan, whose terminal half-life is ~12 h. This agent was administered as a conventional 30-min infusion in the morning or as a chronomodulated infusion from 2:00 a.m. to 8:00 a.m., with peak delivery rate at 5:00 a.m., based on results from experimental studies (48, 66, 77). The interpatient variability of irinotecan and SN-38 exposure was largest in patients given a conventional 60-min infusion in the morning. Conversely, good reproducibility characterized the time curves of drug and metabolite concentrations in patients receiving the chronomodulated infusion, which also produced fewer episodes of severe diarrhea than conventional administration (Figure 7) (150).

Circadian Control of Cellular Determinants of Cancer Chronotherapeutics

Clock genes are expressed at mRNA and/or protein levels in bone marrow, peripheral blood mononuclear cells, circulating leukocytes, oral and colorectal mucosae, skin, heart, liver, lung, breast, ovary, endometrium, abdominal fat, and pineal gland of healthy subjects (151–160). Peak

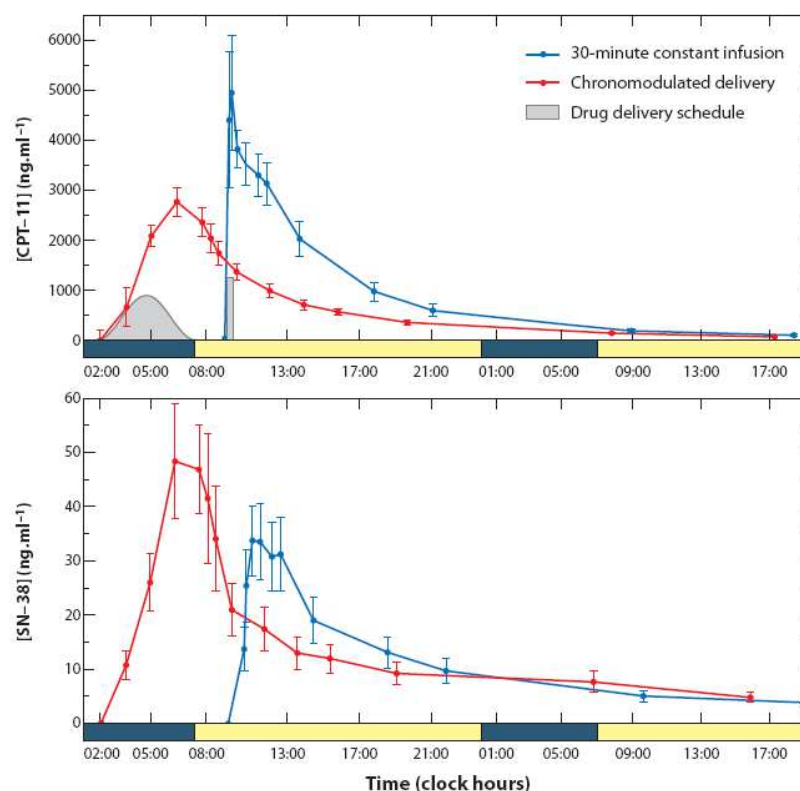


Figure 7

Relevance of circadian timing and chronomodulated delivery for irinotecan pharmacokinetics in cancer patients. Average time courses of plasma pharmacokinetics of irinotecan (CPT-11) and bioactive metabolite SN-38 following a conventional 30-min infusion near 10:00 a.m. or a 6-h chronomodulated infusion with peak flow rate at 5:00 a.m. Results are from a randomized study in 31 patients receiving a fixed dose of 350 mg/m² of irinotecan. Chronomodulated delivery (*a*) reduces mean C_{max} of irinotecan, from 5.53 to 2.91 $\mu\text{g}\cdot\text{ml}^{-1}$ ($p = 0.00012$) and its coefficient of variation from 37% to 17.4%, (*b*) increases the average metabolic ratio (AUC of SN-38:AUC of irinotecan) from 1.89% to 2.53% ($p = 0.02$), and (*c*) reduces the incidence of severe diarrhea from 22.2% to 6% and that of severe asthenia from 44% to 23.5% compared with conventional delivery. Adapted from Reference 189.

mRNA is usually highest in the morning for *bPer1* and *bPer2* and in the evening for *bBmal1*, resulting in similar phase relationships between clock gene transcription patterns in humans and laboratory rodents (157, 160–165). Relevant clock-controlled genes for anticancer drug pharmacology are identified through human circadian transcriptome studies in oral mucosa, mammary epithelium, and adipose tissue (157, 161, 165). Depending on the tissue sampled, the microarray chip used, and the sampling frequency, 4% to 25% of the human genome display circadian variations (157, 161, 165). Gene ontology analysis reveals that most oscillating genes in these differentiated human tissues regulate gene transcription, cell cycle, or metabolism (157, 161, 165). In mononuclear cells of healthy subjects, both *Dpyd* mRNA and activity display a circadian rhythm,

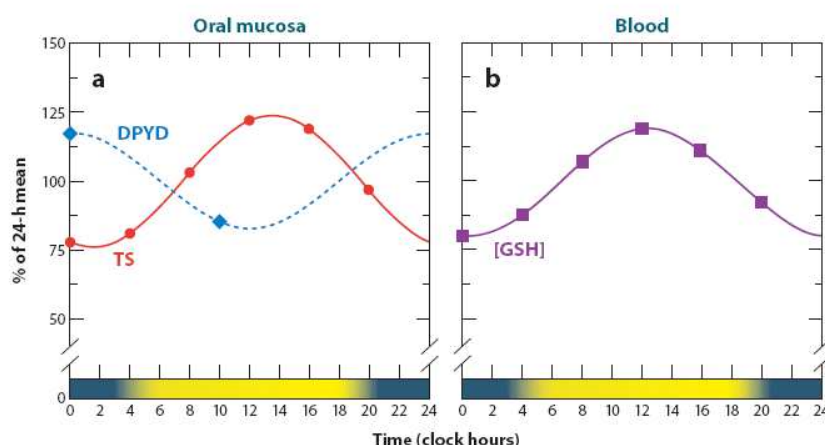


Figure 8

Examples of coordinated rhythmic detoxification and main drug targets in human tissues. (*Left*) Average 24-h sinusoidal estimate of DPYD and TS activities in human oral mucosa, a main target tissue for 5-FU toxicity. DPYD catabolizes 5-FU, whereas TS is the main pharmacologic target of this drug. Adapted with permission from References 163, 168. (*Right*) Average 24-h sinusoidal estimate of GSH concentration in the blood of patients with nasopharyngeal carcinoma. GSH plays an important role in the detoxification of platinum complexes and many other anticancer drugs. Adapted with permission from Reference 175.

*Erratum

with a maximum at night (166, 167). A similar trend for high values at midnight is found for DPYD activity in biopsies of oral mucosa (168). TS activity, but not mRNA, is also rhythmic in this tissue, with a maximum near 1:00 p.m. (163). The apparently opposite phases of DPYD and TS activities in oral mucosa support an increase in the tolerability of healthy tissues for nightly administration of 5-FU (Figure 8).

Plasma GSH concentrations vary by 30% along the 24-h cycle, with a maximum after midnight and superimposed peaks related to cysteine intake during meals (169). Conversely, the highest GSH content occurs in the early morning hours and precedes by ~4 h the peak in S-phase cells in the bone marrow of healthy subjects (170). The protein O⁶-alkylguanine-DNA alkyltransferase removes DNA adducts produced by nitrosoureas at guanine bases. The average O⁶-alkylguanine-DNA alkyltransferase activity in circulating mononuclear cells increased by ~30% from noon to midnight in 12 healthy subjects (35). Anthracyclines, anthracenediones, and epipodophyllotoxins inhibit Top2a, resulting in DNA strand breaks (171). Top2a protein displayed a circadian rhythm with a mean rate of change of 40% and a maximum approximately 7:00 a.m. in the rectal crypt cells of 10 healthy subjects, in synchrony with the peak in DNA synthesis in the same tissue (172).

The cell cycle is synchronously coordinated by the CTS in skin, oral and rectal mucosae, and bone marrow of humans, as in rodents (170, 173). On average, twice as many S-phase myeloid and erythroid cells and bone marrow progenitors are found near 4:00 p.m. compared with midnight in humans (170). The peaks of S-phase cells in the skin and in the oral and rectal mucosae also occur between noon and 4:00 p.m. (173). The circadian organization of cell cycle phases is further demonstrated by consistent 24-h changes in protein markers of cell cycle checkpoints, such as cyclin E (G1/S), cyclin A (G2), and cyclin B1 (M) (162, 173). The rhythmic organization of p53 protein with a peak at 11:00 a.m. and that of *bBd2* mRNA expression with a peak near 1:00 a.m.

DPYD:
dihydropyrimidine
dehydrogenase

FOLFOX:
conventional delivery
schedule associating
5-FU, leucovorin, and
oxaliplatin

further suggest a circadian control of apoptotic pathways in healthy human tissues, as in rodents (33) (G. Bjarnason, personal communication).

Malignant processes can selectively alter some circadian rhythms that drive chronotherapeutic effects in cancer patients. Thus, no consistent circadian rhythm in *Dpyd* mRNA expression was found in a group of 10 patients with advanced gastrointestinal malignancies, a finding at variance with that reported in healthy subjects (166). However, DPYD activity is higher at night than in daytime in patients with gastrointestinal or nasopharyngeal cancer, as measured by enzymatic assays on mononuclear cells or the plasma dihydrouracil:uracil ratio. Thus, the circadian pattern in DPYD activity is similar to that found in healthy volunteers, a finding supporting posttranscriptional control (174, 175) (M. A. Barrat, F. Lévi, & G. Milano, unpublished data). Mean GSH concentration peaked near noon in the peripheral blood of a group of 16 Chinese patients with nasopharyngeal carcinoma (Figure 8) (175). In another group of 15 cancer patients, GSH content did not differ significantly between noon and midnight in bone marrow, yet the proportion of S-phase cells is highest at noon, as it is for healthy subjects (170). Iterative samplings of human cancers through repeat biopsies or cytology aspirations over a 24-h period reveal a circadian organization in some human cancers but not others, as shown for S- and M-phase cells, in individual patients with breast, ovarian, skin, head and neck, or lung cancer or non-Hodgkin's lymphoma (176, 177).

Most investigations in cancer patients, similar to those in healthy subjects, show intersubject variability in circadian waveform, described by periodic components, mesor, amplitude, and phase. However, rest-activity, body temperature, plasma cortisol, and melatonin and circulating blood cell counts, among many circadian biomarkers, display statistically validated and consistent 24-h rhythms in groups of patients with early- or late-stage cancer of the breast, lung, colon, prostate, ovary, or head and neck (176). These findings support the development of standardized cancer chronotherapeutics, with fixed circadian times of administration and fixed drug-delivery chronomodulated profiles for all patients.

Methodology and Results of Clinical Cancer Chronotherapeutics

Over 100 phase I and II clinical trials of cancer chronotherapeutics have involved patients with advanced or metastatic cancer of almost all origins according to a recent PubMed search. Randomized phase III trials have compared chronotherapeutic delivery to a control administration protocol without any time specification. However, patient or hospital convenience makes timing implicit in control treatments, despite the lack of any such stipulation (119, 178). Conversely, constant-rate infusion over at least 24 h eliminates any circadian timing hypothesis for drug administration. Experimental and clinical data show that a constant-rate infusion schedule lasting an integral multiple of 24 h constitutes an adequate control for proof of principle demonstration of cancer chronotherapeutics, if the pharmacologic properties of the drug permit it (Figure 9). This statement is also supported by several mathematical models that include circadian clocks (177, 179, 180).

Chronotolerance in cancer patients. A sequential cross-over design, with toxicity as the main endpoint, takes patients who display severe toxicity on constant-rate or conventional-delivery infusion and then subsequently receive chronotherapeutic administration. For instance, the administration of conventional 5-FU, leucovorin, and oxaliplatin (FOLFOX4) for high-risk colorectal cancer produced grade 3–4 neutropenia in 23 of 68 patients. They then received the same drug doses according to chronomodulated infusion (chronoFLOX1). The chronomodulated delivery schedule produced severe neutropenia in a single patient, despite no prophylactic rh-G-CSF being administered (Table 3) (M. Pirovano, personal communication).

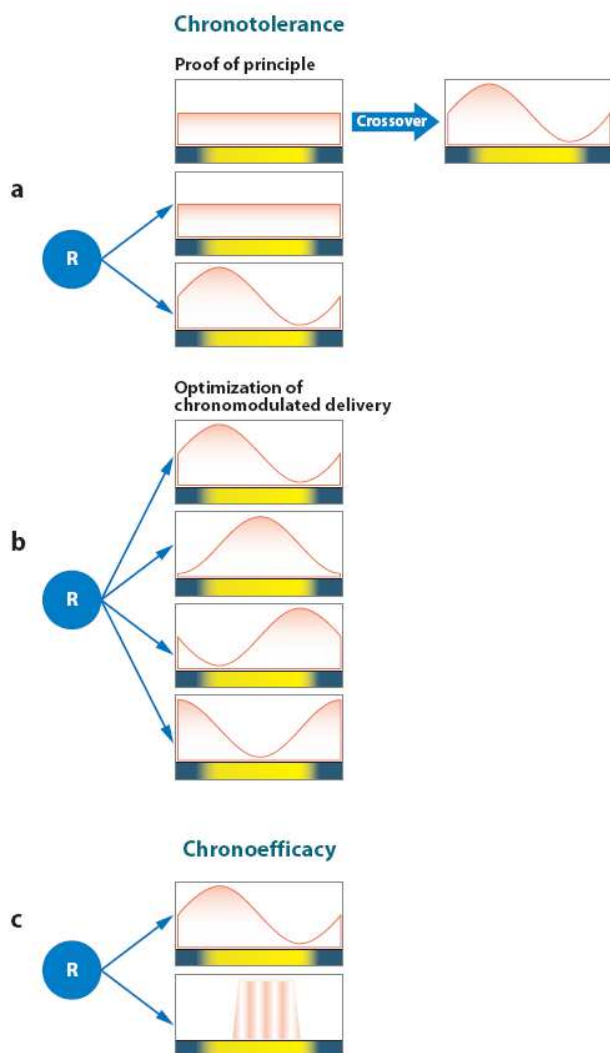


Figure 9

Examples of drug delivery infusion schedules and trial designs used for the clinical validation of standardized cancer chronotherapeutics. See results in Table 3. (a) Proof of principle of chronotolerance, through sequential or randomized trial design (R). (b) Optimization of chronomodulated drug delivery parameters and the relevance of circadian timing for peak drug delivery rate, with tolerability as the main endpoint, using sequential or randomized trial designs. (c) Validation of improved efficacy with randomized comparison with a conventional treatment, which is most often administered between 9:00 a.m. and 5:00 p.m.

Table 3 Main results of comparative clinical trials assessing the role of circadian timing of cancer chemotherapy in comparative trials in patients with colorectal or breast cancer. See text for details on drug delivery schedules.

Trial design	Compared schedules	No. of patients (cancer type)	Main endpoint(s)	Main results	Reference
Cross-over	FOLFOX4 → ChronoFLOX1	68 → 23 (Colorectal)	Gr 3–4 neutropenia	FOLFOX-4, 33.8% ChronoFLO, 4.3%	M. Pirovano, personal communication
Randomized	ChronoFLO5 vs constant rate (flat)	92 (Trial 1, colorectal)	Tolerability Tumor response	(Gr 3–4 mucositis) Chrono, 18% Flat, 89% (Tumor response rate) Flat, 32% Chrono, 53%	(149)
		186 (Trial 2, colorectal)	Tolerability Tumor response	(Gr 3–4 mucositis) Chrono, 14% Flat, 76% (Gr 2–3 sensory neuropathy) Chrono, 16% (Tumor response rate) Flat, 31% Chrono, 51% Flat, 29%	(148)
Time-finding	Eight chronoFLO4 lagged by 3 h	114 (Colorectal)	Tolerability	(Grade 3–4 toxicities) Peak drug delivery: at 4:00 a.m. for 5-FU-LV & at 4:00 p.m. for oxaliplatin, 16.7%; at 4:00 p.m. for 5-FU-LV & 4:00 a.m. for oxaliplatin, 80%	(147)
	Eight chronoVRL lagged by 3 h and fixed chrono5-FU	90 (Breast)	Tolerability	(Leucopenia) Significantly least if peak delivery rate of VRL near 5:00 p.m.	(181)
Randomized	ChronoFLO4 vs FOLFOX2 near MTD	564 (Trial 3, colorectal)	Survival	Similar overall survival. Hazard ratio: Worsened by 38% in females on chrono Improved by 25% in males on chrono	(183)
Randomized	ChronoFLO vs conventional delivery	842 (Meta-analysis of Trials 1–3, colorectal)	Survival per schedule according to gender	Similar overall survival. Hazard ratio (Cox): Worsened by 23% in females on chrono Improved by 23% in males on chrono	(184)

MTD, maximal tolerated dose.

Most multicenter trial designs involve randomized comparisons of a validated chronotherapeutic schedule with constant-rate infusions using the same initial doses over the same treatment duration. Experiments in male mice identified the times of least toxicity near mid-activity for oxaliplatin and near mid-rest for 5-FU (23). These circadian times were extrapolated to cancer patients, with the chronomodulated schedule combining the daily delivery of oxaliplatin over 11.5 h with peak flow rate at 4:00 p.m. and that of 5-FU-leucovorin over 11.5 h with peak flow rate at 4:00 a.m. for 5 consecutive days (chronoFLO5). The other cohort of patients received the same doses of the same three drugs, at a constant rate over the same 5-day span. In two international randomized phase III trials involving 278 patients with metastatic colorectal cancer, chronomodulated delivery reduced the incidence of grade 3–4 mucositis by fivefold and halved the incidence of peripheral sensory neuropathy (**Table 3**) (148, 149). The largest trial also reported a threefold reduction in the rate of hospitalizations for toxic events with chronomodulated infusions (148).

A subsequent study involved the comparison of time-lagged chronomodulated infusion profiles to better define the characteristics of optimal chronotherapeutic delivery (**Figure 9**). Two kinds of multiple-arm chronotherapeutic trials addressed the issue of tolerability as the main endpoint. In the first design, peak times of chronomodulated infusions are lagged over 24 h, yet with fixed intervals between the chronomodulated delivery patterns of the drugs in the combination. In 114 patients with metastatic colorectal cancer, peak times of oxaliplatin and 5-FU-leucovorin shifted by 3 h and compared with the reference profile, where delivery rate peaks at 4:00 p.m. for oxaliplatin and at 4:00 a.m. for 5-FU-leucovorin. This design assumed that it was important to maintain a fixed 12-h interval between the peak delivery rates of oxaliplatin and 5-FU-leucovorin (147). Severe toxicity occurred in 16.7% of the patients on the reference chronoFLO4 schedule and in 80% of those on the opposite chronomodulated modality (**Table 3**) (147). The optimal time of peak delivery rate was defined with its 90% confidence limits at 3:57 a.m. (11:30 p.m. to 9:36 a.m.) for 5-FU-leucovorin and at 3:57 p.m. (11:30 a.m. to 9:36 p.m.) for oxaliplatin. Such chronotolerance was confirmed for carboplatin and 5-FU-leucovorin in a randomized trial involving 45 patients with advanced non-small-cell lung cancer, receiving the three-drug chronomodulated schedules with peak drug delivery rates shifted by 8 hours (147). Patients treated with the reference profile experienced less frequent severe toxicity (6.7% versus up to 40%) and less frequent treatment delays or dose reductions (**Table 3**) (147).

Another design to find optimal times of administration involves the staggering of peak times of chronomodulated delivery of the single drug of interest every 3 or 4 h over 24 h (181). The other drugs in the combination are administered according to a fixed chronomodulated schedule. This results in varying intervals between the phases of the drug delivery profiles. In 90 patients with metastatic breast cancer, the peak delivery time of vinorelbine was shifted by 3, 6, 9, 12, 15, 18, or 21 hours, whereas peak delivery time of chronomodulated 5-FU was fixed at 4:00 a.m. (181). Estimated least leukopenia corresponds to peak vinorelbine delivery at 5:15 p.m. (2:12 p.m. to 8:08 p.m.), in good agreement with chronotolerance in female mice (182). Fewer dose reductions and/or treatment delays occurred for peak vinorelbine delivery at 8:13 p.m. (6:07 p.m. to 10:39 p.m.) (**Table 3**). Late evening vinorelbine tended to minimize the occurrence of severe neutropenia, febrile neutropenia, and gastrointestinal toxicities only at the higher dose tested (181). Both trial designs assume that the CTS and the clock-controlled pharmacologic pathways remain stable after being challenged by the first medication studied. However, vinorelbine can induce circadian disruption in mice (102). Similar designs can also help identify the optimal infusion duration, flow rate amplitude, and number of treatment days per course. The incorporation of translational endpoints is essential for the identification of patient subgroups and their corresponding distinct optimal chronomodulated schedules.

Relevance of chronomodulated delivery for efficacy. The relevance of a validated chronomodulated delivery regimen for antitumor efficacy was investigated in patients with metastatic colorectal cancer using tumor response rate and survival as the main criteria. Two consecutive European randomized trials compared chronoFLO5 with constant-rate infusion over 5 days every 3 weeks in a total of 278 patients. The percentage of patients whose metastases regressed by $\geq 50\%$ was 29% on constant-rate infusion and 51% on chronomodulated delivery ($p < 0.001$). However, overall survival did not significantly differ according to treatment schedule (17, 148, 149). A third randomized trial compared the chronomodulated administration of the same three drugs over 4 days (chronoFLO4) with a 2-day conventional administration schedule without any timing stipulation (FOLFOX2) in 564 previously untreated patients with metastatic colorectal cancer (183). The trial was intended to treat each patient at the near maximum tolerated dose. Overall survival, the main endpoint in this large international study, did not differ as a function of treatment schedule. However, the relative risk of an earlier death on chronoFLO4 significantly increased by 38% in women and significantly decreased by 25% in men compared with conventional delivery (183). A recent meta-analysis of these three randomized trials in 842 patients with metastatic colorectal cancer confirms that the three-drug chronomodulated infusion achieves similar or worse efficacy compared with conventional delivery in women. In men, however, the same chronoFLO treatment significantly increases tumor response and survival compared with conventional delivery, independent of other prognostic factors (Table 3) (184).

Three hypotheses are currently being tested to account for such gender-schedule interactions: (a) the occurrence of a different circadian genotypic profile between males and females with colorectal cancer, (b) the sex dependency of circadian pharmacology of anticancer drugs, and (c) the occurrence of excessive toxicity in women causing circadian disruption, and thus the impairment of chronotherapeutic mechanisms. This hypothesis is supported by the occurrence of 20% to 50% more toxicities in women (147, 183). Preliminary studies show that cancer treatments can disrupt the rest-activity rhythms in cancer patients, and this disruption is associated with systemic toxicities (185).

TOWARD THE PERSONALIZATION OF CANCER CHRONOTHERAPEUTICS

The standardization of cancer chronotherapeutics has been mostly developed using male B6D2F1 mice and successfully transferred to the clinic. However, clinical and translational data also show differences in circadian rhythms of individual cancer patients that are relevant for therapeutic outcomes. Dedicated *in vitro*, *in vivo*, and *in silico* experimental models and technologies are paving the way to personalized cancer chronotherapeutics.

Circadian Physiology and Clock Genes in Cancer Patients

Circadian alteration or disruption of plasma cortisol and melatonin, blood cell count, liver enzymes, or renal tests were observed in individual patients (176, 186). The patients with near normal cortisol rhythm had synchronous circadian rhythms in bone marrow S-phase cells and 5-FU concentrations during constant-rate infusion for 24 h. In contrast, the patients with damped or ablated cortisol rhythm displayed blunted, if any, rhythmic patterns in bone marrow or plasma 5-FU concentrations (170, 187). Minimally invasive techniques, such as rest-activity monitoring or iterative salivary cortisol determinations, provided CTS estimates in large populations of cancer patients that match those routinely treated for cancer (12, 13). Nearly a third of patients with metastatic breast or colorectal cancer displayed poor rhythms in salivary cortisol and/or rest-activity before

they received chemotherapy (11–13). Moreover, circadian disruption appears as an independent prognostic factor of survival (11–13) and hinders both chronotherapeutic mechanisms and host control of malignant processes.

Human clock genes are highly polymorphic, as documented by large population-based studies (188, 189). The phenotype associated with germline variants of clock genes can affect not only sleep preferences, mood disorders, and metabolic diseases but also cancer risk. Extreme circadian rhythm-related sleep disorders, such as familial advanced or delayed sleep syndromes are caused by polymorphisms in *Per2* and *Csnk1d*, and *Clock* and *Per3* genes, respectively (189, 190). Less extreme chronotypes of morningness or eveningness preferences seem to be also more frequently associated with genetic variants of *Per2*, *Per3*, *Clock*, and *Csnk2a2* (189, 190). Polymorphisms of the clock gene *Npas2*, a *Clock* homolog that predominates in specific tissues, are associated with a decreased risk of non-Hodgkin's lymphoma and breast and prostate cancers (191–193). Conversely, *Cry2* polymorphisms are associated with an increased risk of non-Hodgkin's lymphoma and prostate cancer (191, 194). A limited study in patients with esophageal cancer found no significant association between 5-FU circadian pharmacokinetics and a *Clock* gene polymorphism, reported to affect the time course of antidepressant-induced insomnia (195, 196). Polymorphisms in tissue-specific clock-controlled genes can also account for interindividual differences in relevant circadian rhythms, as shown for plasminogen activator inhibitor type I (PAI-1) that circulates rhythmically with a morning peak in human peripheral blood. Genomic variants within the gene promoter region critically determine 24-h changes of plasma concentrations of PAI-1, from severely blunted to severalfold (197). Similarly, rhythmic patterns in locomotor activity and temperature rhythms are affected by polymorphisms in a serotonin reuptake transporter in depressive patients (198, 199).

In human tumors, the mRNA or protein expression of the clock genes *Per1*, *Per2*, or *Per3* as well as *Npas2* or *Dec1* is markedly decreased on average or deregulated in comparison with reference tissues. This is the case for cancers of the breast, lung, colon, endometrium, ovary, pancreas, and bone marrow, a finding supporting frequent circadian disruption in human malignancies (32, 34, 152, 200). The altered expression of clock genes in human tumors can influence the efficacy of cancer chronotherapeutics. The mRNA expression of *Per1* and *Dpyd* are strongly correlated in primary human colorectal cancers, and more so in women. The relation between *Per1* and *Dpyd* expression is disrupted in poorly differentiated colon carcinoma cells, possibly resulting in the suppression of tumor *Dpyd* oscillations (200).

Chronopharmacology at the Cellular Level

The circadian transcriptome has been determined using microchip DNA arrays on iterative samples from synchronized cultures of mouse or rat fibroblast or immortalized SCN cell lines (201, 202). Rhythmic mRNA expression is demonstrated in cell cultures for genes encoding for transcription factors, such as *Dbp*, cell cycle, apoptosis, differentiation, glucose metabolism, and detoxification, with tissue specificity (201, 202). Synchronized cell cultures display rhythmic transcription of drug metabolism and targets, such as *Cyp2e1*, *Cyp3a4*, and *Top1* (90, 203, 204). Ex vivo cell cultures of bone marrow progenitors from male B6D2F1 or Balb/C mice show sustained circadian rhythms in pharmacodynamic response to granulo-monocytic colony-stimulating factor (GM-CSF) over 4 days (205). The circadian maximum in the proliferative response of bone marrow cells to GM-CSF occurs at the same circadian time in vitro and in vivo (205). Ex vivo bone marrow liquid cell cultures are also synchronized with serum shock and thus can be used for investigating cellular chronopharmacology in hematopoietic cells (206). The circadian period and amplitude of luminescent-reporter human osteosarcoma cells are novel clock-related endpoints that can be used to screen for pharmacologically active compounds (207). The lengthening of

the circadian period has been shown to be a novel pharmacologic property of seliciclib, a finding confirmed by our team in mice with a free running CTS. The colon adenocarcinoma cells Caco-2 express clock genes and proteins in synchronized cell cultures (158). Caco-2 cells are also used as an in vitro model to investigate pharmacokinetic-pharmacodynamic (PK-PD) relations of anticancer drugs (158, 208, 209). Synchronized undifferentiated Caco-2 cells display coordinated circadian transcription patterns of the clock genes *Rev-erba*, *Per2*, and *Bmal1* and the irinotecan pharmacology genes *Ces2*, *Ugt1a1*, *Abcb1*, *Abcc1*, *Abcc2*, *Abcg2*, and *Top1* (Figure 10) (210). Such an in vitro system allows the dynamic determination of chronoPK-PD relations for building

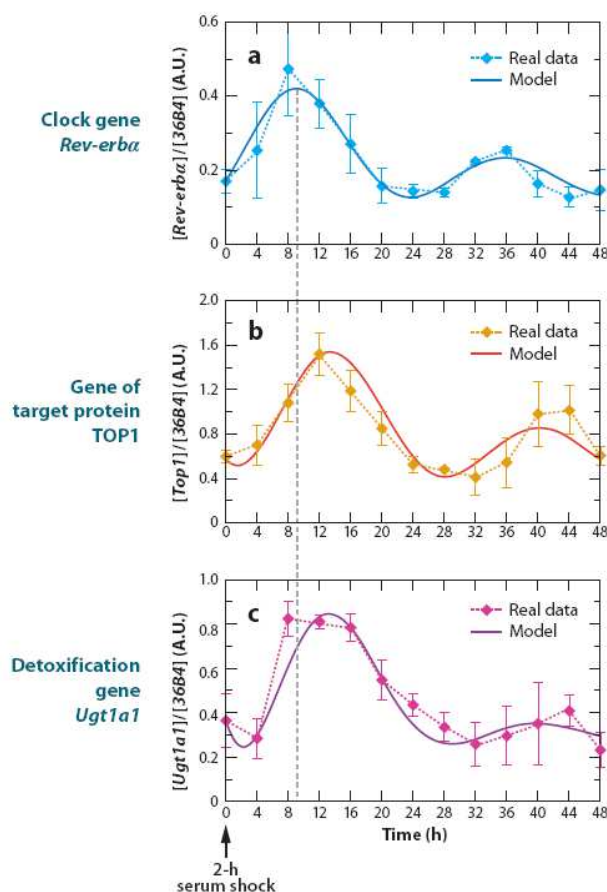


Figure 10

Synchronized in vitro model of cellular determinants of irinotecan chronopharmacology. Temporal changes of mRNA expression of (a) *Rev-Erba*, (b) *Top1*, and (c) *Ugt1a1* in undifferentiated Caco-2 cells over the 48 h following synchronization of cell cultures with 2-h serum shock (vertical arrow). Actual data have been normalized to housekeeping gene 36B4 (dotted lines) and output functions from mathematical model incorporating circadian clocks and decay functions (solid lines).

first-generation chronotherapeutic models at the cellular level, with the perspective of personalizing cancer chronotherapeutics.

The properties of individual human circadian clocks can be determined using *ex vivo* fibroblasts from skin biopsies following transformation with lentiviral *Bmal1-luciferase* reporter (211). The continuous monitoring of *Bmal1* transcription with luminescence detectors reveals that the circadian period of *Bmal1* differs from 24.3 ± 0.4 h in the subjects with an early chronotype to 24.7 ± 0.3 h in those with a late chronotype (211).

Chronotoxicity Classes in Mouse Models

The pharmacologic effects of anticancer drugs differ largely according to cell lines and species, strain, or gender of experimental animal models. The heterogeneity of cancer cells and cancer tissues is an additional cause of variability of anticancer drug effects. For instance, strain-specific differences in glucuronidation reactions and irinotecan detoxification characterize C57/Bl6, DBA2J, and BALB/c mice and result in strain-dependent toxicity and efficacy of this drug (81). The overall tolerability of theprubicin in B6D2F1 mice is approximately threefold better in females than in males. Moreover, the optimal circadian timing for tolerability occurs at ZT10 in males and at ZT14 in females. The magnitude of chronotolerance for the highest dose tested is 8-fold in male and 0.6-fold in female B6D2F1 mice (F. Lévi, unpublished data). In contrast, the optimal circadian timing of theprubicin is located at ZT15 in male C57/Bl6 mice (46). Optimal circadian timing improves cisplatin tolerability threefold at ZT19 in female F344 Fischer rats and twofold at ZT15 in male B6D2F1 mice compared with dosing 12 h apart (59, 212). Not only the average level of irinotecan tolerability but also its circadian pattern differ significantly as a function of mouse strain and sex. Optimal circadian timing of irinotecan is at ZT11 in male and at ZT15 in female B6D2F1 mice. The highest plasma AUC of CPT-11 and SN-38 results from irinotecan administration at the most toxic time in female B6D2F1 mice (82). However, no such consistent relationship between circadian pharmacokinetics and toxicity is found in male mice of the same strain (82). The identification of underlying molecular and physiological circadian determinants of distinct chronotoxicity classes may subsequently guide the clinical development of tailored cancer chronotherapeutics.

NOVEL INSIGHTS INTO CANCER CHRONOTHERAPEUTICS THROUGH MATHEMATICAL MODELS

The Optimization of Chronotherapeutic Delivery under Chronotoxicity Constraints

Mathematical models of cancer chronotherapeutics are designed with the goal of therapeutic optimization. Just as cell population dynamics models are used to represent tumor and healthy tissue kinetics, optimization consists of finding the best possible infusion time schedule (solution to an optimal therapeutic control problem) that can be used to minimize a population of cancer cells: its absolute minimum, or in contrast its maximum within a treatment window, if one only wants to stabilize rather than eradicate the tumor. This minimum or maximum is the objective function to be minimized by therapeutic control. Optimization is always performed under constraints. The main constraint by far is to respect host tolerance for anticancer medications. This requires the definition and the quantification of the healthy cell populations to be shielded from toxic insult, using parameters that depend both on the drug and on the tissues turning over quickly, such as bone marrow, gut, and skin. Other constraints such as maximum total dose and maximal drug

infusion flow are easily defined and are usually the only ones that are considered in conventional chemotherapeutics, yet they are inappropriate for cancer chronotherapeutics.

For such a treatment strategy, one needs to consider toxicities in a dynamic way, by defining infusion schedules that vary along the circadian time span. A toxic threshold for the healthy target tissue is physiologically defined, not as a drug concentration or dose, but as a lower-limit cell population number under which it would be hazardous (potentially lethal) to descend. Such a limit parameter drives the adaptation of the drug infusion flow rate in individual patients. To accurately define such a limit and adapt it to the delivered drug infusion flow, cell population dynamics models are designed with a physiological structure, that is, with respect to cell cycle progression, cyclin concentrations, etc., including control targets, to allow the representation of the mechanisms of drug toxicity (213).

This formulation of the chronotherapeutic optimization problem in terms of objective and constraint functions allows the use of mathematical methods such as optimal control on a physiological, not only an empirical, basis. This is illustrated in a proof of principle study of in silico chronotherapeutics with oxaliplatin. Parameter identification is performed on tumor growth curves in mice, with a simplified PK-PD model based on the jejunal toxicity and the antitumor efficacy of oxaliplatin (61, 66, 80, 177, 180). The solution to the optimal control problem is a theoretically optimized drug infusion flow (Figure 11). The treatment constraints critically determine the optimal chronotherapeutic schedule. Interestingly, constant rate infusions always achieve worse therapeutic outcomes than optimized chronomodulated regimens in these models. The same principles are currently driving the in silico optimization of chronomodulated drug delivery schedules to be administered with multichannel programmable pumps in cancer patients.

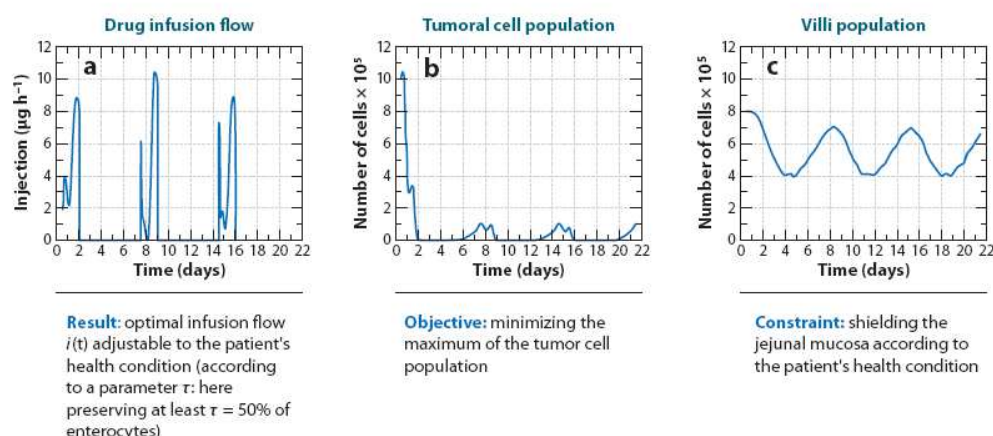


Figure 11

Relevance of optimal control theory for designing chronotherapeutic delivery schedules. Results of an optimal control problem using a pharmacokinetic-pharmacodynamic model of single agent oxaliplatin for parameter identification in tumor-bearing mice.

(a) Numerical solution to the optimal control problem resulting in an optimized chronomodulated drug delivery schedule.

(b) Dynamics of the tumor cell population under the objective function of minimization of its local maxima. (c) Dynamics of the healthy cell population under the constraint of keeping it above a given threshold. Adapted from Reference 234.

Relation Between Chronotoxicity and Chronoefficacy

Chronotoxicity and chronoefficacy involve similar molecular mechanisms at the cell level, and thus can be represented in the same way by the action of drugs on molecular cell targets. However, anticancer drugs can elicit different responses in healthy or malignant cell populations. Physiologically based PK-PD mathematical models help us understand the molecular mechanisms that discriminate the differential circadian response of healthy cells and cancer cells to anticancer drugs. Thus, *in silico* testing can probe the respective relevance of determinants of cell cycle control, apoptosis, and DNA repair in cell population dynamics, including the role of p53 mutational status, but they can also be found in drug-processing cell mechanisms, including reduced glutathione and its synthesis, and specific enzymes, such as DPYD or UGT1A1. Another important discriminating property of cancer cells is their ability to express ABC transporters, including ABCB1, that transport xenobiotics from inside the cell to the outside. Many of the mechanisms mentioned above show circadian modulation of their activity, at least in normal cells, so that poor or absent sensitivity to CTS inputs in cancer cells could account for observed differences of responses to time-scheduled drug regimens with respect to chronoefficacy and chronotolerance.

The differences in drug response with respect to host and tumor circadian clocks, which are intended to be exploited by optimized chronotherapeutic schedules, may thus be due to the disruption of mechanisms at the individual cell level, but they may also be due to disruption of the physiological synchrony with respect to phases of the cell division cycle in normal proliferating cell populations (e.g., gut, bone marrow). From observations on circadian clock-controlled gene expression in tumors and in healthy tissues, it has been hypothesized that (*a*) the CTS is an essential coordinator, and (*b*) poor circadian synchronization offers a proliferation advantage for cancer cell populations over well-synchronized healthy cell populations. Although no causal relationship between poor synchronization and enhanced proliferation has been established experimentally, the desynchronization between tumor cells and healthy cells provides possible insights into the relation between chronoefficacy and chronotoxicity (177, 179, 214). The use of a cellular automaton model reveals that strong circadian synchronization among cells is an essential feature for healthy cell populations in order to display a phase of low susceptibility to cytostatics. In contrast, cancer cells, with blunted synchronization, remain sensitive to the action of the drug with low variation in susceptibility. This alone might explain the differential response of healthy and malignant cell populations to optimally tolerated chronomodulated chemotherapy (214). The model further reveals that the cell cycle duration, which can differ between tumor and healthy cell populations, but also among individual tumor cells, is another critical parameter (214). Taking into consideration both cell synchronization with respect to cell cycle phases and cell cycle duration, theoretically optimized infusion schemes, which should achieve the best tumor containment and best preservation of healthy cells, can be generated.

The improved knowledge regarding time determinants in the physiological mechanisms at work (drug detoxification, cell cycle control, cell population synchrony) is bringing more flexibility to the dynamic delivery of cancer treatments to individual patients. The shielding of the patient's CTS from treatment-induced disruption represents a new dynamic constraint that could be prevented through adequate drug delivery patterns, early detection through dedicated technology, and rapid correction through feedback into chronotherapeutic algorithms to further optimize chronotherapeutic patterns along the course of cancer treatments in individual patients.

Mathematical Models and Technologies for Tailoring Chronotherapeutic Delivery

Mechanistic models can include any relevant intracellular mechanism involved such as polymorphisms of genes and the dynamic organization and treatment responses of cells, tissues, and whole organisms. For example, genetic differences in 5-FU catabolism by DPYD can be taken into account by different K_m and V_{max} values of the enzyme activity (Figure 12). Physiologically based mathematical models designed to propose theoretically optimized chronotherapeutic delivery schedules must consist of (215):

1. Dynamic models of cell populations, physiologically structured, with structure variables describing evolution in the cell-division cycle for both healthy and cancer cell populations (with different parameters) and with prescribed targets (parameters of the population dynamics model) for circadian and pharmacological control (213).
2. Molecular chronoPK-PD models for anticancer drugs, including action exerted on their targets in cell population models (pharmacodynamics), intracellular transformation of drugs by enzymes, active efflux proteins or other intracellular agents (intracellular pharmacokinetics), and drug fate from its infusion in the general circulation, possibly via previous intestinal absorption, and certainly with hepatic detoxification, until its delivery to peripheral cells, thus calling for the design of a whole-body compartmental chronoPK model.
3. Optimal control methods of chronomodulated drug infusion rates dealing with several drugs administered simultaneously (180). These methods handle several targets on cell population dynamics, one objective function to be minimized (i.e., a measure of the tumor cell population), and constraints that involve (a) at least preservation of an absolute number of the healthy cell population that is the target of toxicity, (b) prevention of the occurrence of a resistant tumor cell clone, and (c) preservation of functional circadian clocks.

CONCLUSION AND PERSPECTIVES

Through its multilevel hierarchical organization, the CTS controls the metabolism, detoxification, and pharmacodynamic effects of anticancer drugs of all pharmacologic classes, both in experimental models and in cancer patients. Common features characterize the CTS and clock-controlled pathways relevant for cancer chronotherapeutics in living beings. Monitoring of circadian rhythms and programmable-in-time drug delivery technologies enable the translation of the

Figure 12

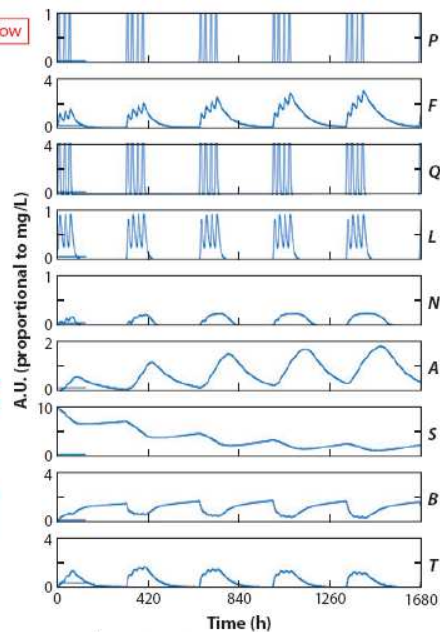
Mathematical model combining intracellular molecular chronopharmacokinetics and chronopharmacodynamics of 5-FU and leucovorin (LV) on the intracellular target enzymethymidilate synthase (TS). (a) In this system of ordinary differential equations, where dynamic variables are concentrations, drug inputs i and j are the plasma infusion flows of 5-FU and LV, respectively, and the pharmacodynamic output is the blockade of TS under the form of a stable ternary complex, secondarily degraded, but which irreversibly consumes free TS when it is formed. The intracellular active compounds, FdUMP for 5-FU and methylene tetrahydrofolate (MTHF) for LV, exert their action on TS by yielding first a reversible binary complex B binding 5-FU and TS, and then the irreversible ternary complex T by the adjunction of MTHF (pharmacodynamic part: Equations 7, 8, 9). The intracellular pharmacokinetic part of the model, in addition to simple transport and intracellular transformation for LV (Equations 3 and 4), describes (Equation 1) the degradation of 5-FU (variable P) in the liver, considered as a filter inside the plasma compartment, by hepatic dihydropyrimidine dehydrogenase (DPYD) and, through a saturable mechanism, its entry in the cell, where (Equation 2), under the form of FdUMP (variable F), either it is expelled by an FdUMP-triggered—via a nuclear factor (variable N)—ABC transporter (variable A), as represented in Equations 5 and 6, or it binds to free TS (variable S). Hepatic DPYD and intracellular TS are represented with their circadian rhythms by a cosine-like modulation. (b) The physiological basis of the variables considered is illustrated by a symbolic representation of the plasma compartment (tubular) where drug inputs i and j are infused and that of the intracellular compartment (*ellipse*) where biochemical reactions occur, involving physiological and pharmacological compounds.

a

- 1 $\frac{dP}{dt} = -k_0 P - \frac{aP}{b+P} - I_{DPYD} \frac{P}{m_{DPYD} + P} + \frac{i(t)}{V}$ Input i = 5-FU infusion flow
- 2 $\frac{dF}{dt} = \frac{a}{\xi} \frac{P}{b+P} - \frac{AF}{c+F} - k_1 FS + k_{-1} B$
- 3 $\frac{dQ}{dt} = -k_2 Q + \frac{j(t)}{V}$ Input j = LV infusion flow
- 4 $\frac{dL}{dt} = \frac{k_2}{\xi} Q - k_3 L - k_4 BL$
- 5 $\frac{dN}{dt} = \frac{\kappa F^n}{\lambda^n + F^n} - \mu N$
- 6 $\frac{dA}{dt} = \mu N - \nu A$ A = ABC transporter (active drug efflux)
- 7 $\frac{dS}{dt} = -k_1 FS + k_{-1} B + \theta_{TS} (S_0 - S)$ S = free thymidylate synthase (TS)
- 8 $\frac{dB}{dt} = k_1 FS - k_{-1} B - k_4 BL$
- 9 $\frac{dT}{dt} = k_4 BL - \nu_T T$ Drug output T = blocked thymidylate synthase (stable ternary FdUMP-MTHF-TS complex)

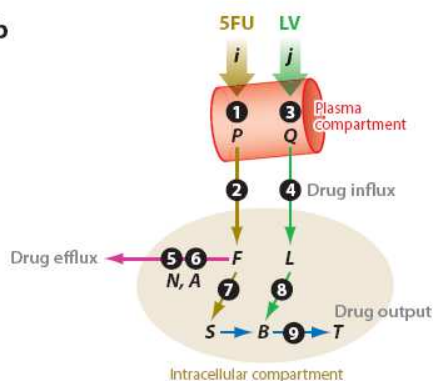
where $I_{DPYD} = I_{DPYD_BASE} \left\{ 1 + \varepsilon \cos \frac{2\pi(t - \phi_{DPYD})}{24} \right\}$

and $S_0 = S_{0_BASE} \left\{ 1 + \delta \cos \frac{2\pi(t - \phi_{TS})}{24} \right\}$



P = Plasma (5-FU)
 F = Intracellular (FdUMP)
 Q = Plasma (LV)
 L = Intracellular (MTHF)
 N = 5-FU-triggered nuclear factor
 A = ABC transporter activity, nuclear factor-induced
 S = Free (TS) (not FdUMP-bound)
 B = (FdUMP-TS) reversible binary complex
 T = (FdUMP-TS-LV) stable ternary complex

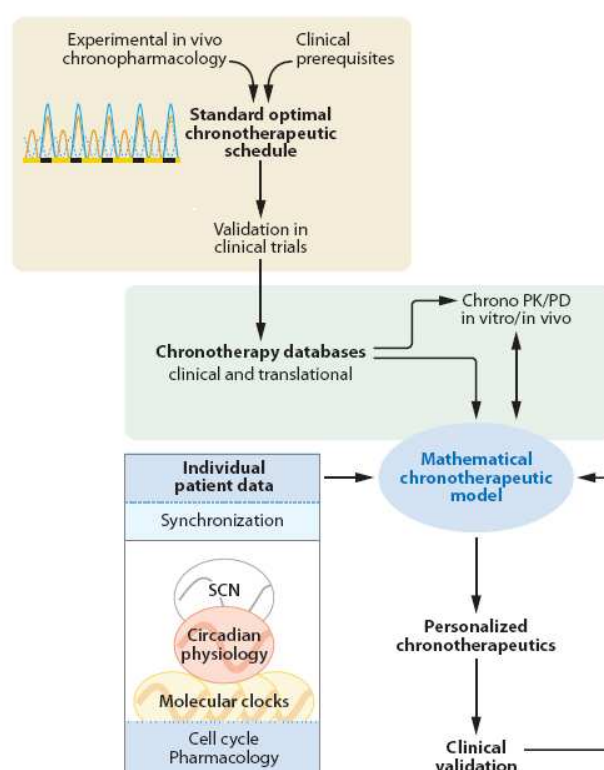
b



severalfold improvement of the tolerability and efficacy of cancer treatments from experimental models to cancer patients. Standardized chronotherapeutics involves the administration of anti-cancer drugs with fixed chronomodulated profiles and fixed circadian timing for all the patients. Adequate chronomodulated schedules usually reduce interpatient variability in drug pharmacokinetics compared with conventional administration of drugs. The clinical development of standardized chronotherapeutics was the first to reveal the safety and antitumor activity of oxaliplatin in patients with metastatic colorectal cancer, to trigger a new medical and surgical strategy with curative intent for this disease, and to specify the need for chronomodulated infusion protocols for nonhospitalized patients, with persistent circadian synchronization. However, CTS components can be variably altered among cancer patients, despite consistent group patterns in most circadian biomarkers. These observations call for the definition of subgroups of patients with distinct circadian characteristics.

After decades of therapeutic development ruled by the standard dosing regimens, the personalization of treatment becomes a central goal in the field of cancer. However, the tools and methods to achieve such a goal remain elusive. Database mining and translational circadian studies of cancer patients, involving physiological, pharmacogenetic, and pharmacogenomic endpoints are required (Figure 13). These translational investigations need to be guided by new dedicated experimental models. Thus, the circadian map of the molecular mechanisms of anticancer drug

Figure 13
Main steps in developmental chronotherapeutics of cancer, from standardization to personalization.



chronopharmacology currently helps in building theoretical chronotherapeutic models at cellular, tissue, and whole organism levels. Chronopharmacokinetic and chronopharmacodynamic data from synchronized cell cultures and from mice of different genders and strains are being integrated to design distinct optimal chronotherapeutic patterns for anticancer drug delivery according to CTS status and dynamics (<http://www.biosim-network.net/> and <http://www.chrono-tempo.org/>). The paradigm of personalized chronotherapeutics for cancer is stimulating the development of novel cancer treatment algorithms both for chronomodulated drug delivery and for the selective targeting of defective circadian clocks or clock-controlled pathways.

Technological advances now allow for complex drug delivery after both systemic and oral administration routes through programmable pumps and oral multiple-unit preparations (216–218). Novel drug delivery systems could enable the personalization of chronotherapeutics with oral anticancer drugs through patient- and drug-specific preparations, thus contributing to improvement of the currently limited tolerability and efficacy of these agents.

The acquisition of relevant temporal information in individual patients through dedicated technologies will enable a systems chronopharmacology approach to optimally adjust the dynamic patterns of anticancer drug exposure to the circadian timing system of the individual cancer patient.

SUMMARY POINTS

1. The endogenous circadian timing system rhythmically controls cellular metabolism and proliferation, which determine the pharmacologic effects of anticancer agents.
2. Circadian timing significantly modifies tolerability and efficacy in experimental models and in cancer patients.
3. Mechanisms involve the circadian control of phase I and II metabolism and that of cell cycle checkpoints and apoptosis.
4. Optimal circadian timing and dosing of anticancer drugs can differ according to gender.
5. Studies in male mice translate into large and significant improvements in tolerability and efficacy in male patients with cancer.
6. In vitro, in vivo, and in silico models of cancer chronopharmacology are leading toward the personalization of cancer chronotherapeutics.

FUTURE ISSUES

1. In vitro models of anticancer drug chronopharmacology need to be developed and diversified.
2. Mathematical models will integrate the reciprocal signaling between circadian clocks and drug metabolism, cell cycle, DNA repair, and apoptosis in healthy and cancer cells through systems biology approaches.
3. Multiple preclinical models with distinct clock properties are required for the personalization of cancer chronotherapeutics and the prediction of optimal chronomodulated drug delivery.
4. The stages where chronotherapeutics will be integrated into the development of new anticancer drugs will have to be defined, ranging from screening to clinical phases.

5. Dedicated diagnostic technologies are needed for dynamic quantitative and noninvasive assessment of circadian timing system components.
6. Multiple dedicated drug delivery technologies will enable the ambulatory administration of personalized cancer chronotherapeutics.

DISCLOSURE STATEMENT

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Chronothérapie et horloge moléculaire: implications cliniques en oncologie.

Innominato PF, Lévi FA, Bjarnason GA.

Le système circadien rythme le métabolisme des médicaments ainsi que plusieurs étapes du cycle cellulaire, de la réparation de l'ADN, de l'apoptose et de l'angiogenèse, dans les tissus sains et cancéreux. Des études réalisées chez les rongeurs et chez l'Homme ont montré que la toxicité et l'activité antitumorale des agents anticancéreux peuvent être modifiées de façon significative par leur horaire d'administration. Les altérations du rythme d'activité-repos, un biomarqueur du système circadien, sont fréquents chez les patients atteints de cancer, et davantage encore après administration des médicaments anticancéreux à l'heure de toxicité maximale. La disruption du système circadien accélère la croissance tumorale. Les relations complexes entre le système circadien de l'hôte, le cancer et ses traitements font aussi intervenir d'autres facteurs, tels que le sexe, le mode de vie, la génétique, et notamment, les polymorphismes et/ou le niveau d'expression des gènes de l'horloge. La prise en compte du stade circadien d'administration est importante pour toutes les phases du développement d'une nouvelle molécule afin d'optimiser l'index thérapeutique des nouveaux médicaments anticancéreux. L'identification des paramètres critiques de la biologie circadienne de l'hôte et de la tumeur chez chaque patient permettra de personnaliser les schémas chronomodulés d'administration, optimisant ainsi le bénéfice chronothérapeutique.



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ABSTRACT

The circadian timing system drives daily rhythmic changes in drug metabolism and controls rhythmic events in cell cycle, DNA repair, apoptosis, and angiogenesis in both normal tissue and cancer. Rodent and human studies have shown that the toxicity and anticancer activity of common cancer drugs can be significantly modified by the time of administration. Altered sleep/activity rhythms are common in cancer patients and can be disrupted even more when anticancer drugs are administered at their most toxic time. Disruption of the sleep/activity rhythm accelerates cancer growth. The complex circadian time-dependent connection between host, cancer and therapy is further impacted by other factors including gender, inter-individual differences and clock gene polymorphism and/or down regulation. It is important to take circadian timing into account at all stages of new drug development in an effort to optimize the therapeutic index for new cancer drugs. Better measures of the individual differences in circadian biology of host and cancer are required to further optimize the potential benefit of chronotherapy for each individual patient.

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1. Introduction

1.1. The circadian timing system

The circadian timing system is a hierarchical network that temporally coordinates biological and physiological processes along the 24-h day [1–11]. Endogenous, self-sustained, 24-h rhythmic oscillations within the hypothalamic pacemaker, the paired supra-chiasmatic nuclei, are entrained to the 24-h changes in external environment through input signals from sensory organs and from other brain areas [1–10]. In turn, the hypothalamic pacemaker generates behavioral rhythms and synchronizes ubiquitous clocks in peripheral organs through neuronal, physiological and endocrine output signals, resulting in measurable and therapeutically exploitable circadian variations [1–8,10,12–14]. Thus, rhythms in hormonal secretions including cortisol, catecholamines and melatonin, autonomic nervous system activity, core-body temperature, physical and cognitive performance (Fig. 1) form a dynamic physiological network which resets and coordinates the peripheral molecular clocks. These

rhythms can be monitored and serve as biomarkers of the circadian timing system [1–8,12–15].

Each mammalian cell is equipped with a self-sustained molecular clock, resulting from interconnected and auto-regulatory transcriptional, post-transcriptional and post-translational loops involving a genetic network of at least 15 gene products [1,13,16–25]. Alongside these identified core clock genes, at least 10 other proteins, including several kinases and the proteasomal machinery, are implicated in the control of clock protein stability and degradation, thus regulating the abundance, activity and subcellular localization of the core clock proteins [1,13,16–25]. This molecular clock generates self-sustained ~24-h cyclic oscillations in individual cells, which can be entrained by internal cues, such as endocrine and temperature rhythms [1,10,13,16–25]. One of the main outputs of the ticking molecular clock within each cell is the coordinated transcription of ~10% of the genome with a circadian rhythmic pattern [1,10,16,25–35]. The circadian transcriptome is tissue-specific, and constitutes the molecular basis of circadian rhythms in the whole organism. Clock genes expression at the mRNA and/or protein levels has been reported in 17 tissues of healthy human subjects and/or cancer patients [1,36–67] (Table 1).

This orchestration is controlled by specific activation of the transcription of clock-controlled genes by core clock proteins [30,31,68] (Fig. 2). Moreover, several of the clock-controlled genes have themselves properties of transcriptional factors, thus amplifying the oscillatory signals generated by the molecular clock, acting through other specific promoters [30,31,68] (Fig. 2). Clock-controlled genes are not only limited to protein-encoding sequences, since further fine-tuning of regulation and tissue specificity of the circadian transcriptome and its associated circadian proteome is provided by cyclic variations in microRNA (miRNA) expression and regulation of translation [69–81]. Another level of coordinated circadian transcriptional regulation relies on the chromatin remodeling by core clock proteins through histone modifications, producing dynamic changes in chromatin transitions [33,82–84].

1.2. Relevance of circadian perturbation in cancer

Large epidemiologic studies have shown a significant association between circadian disruption induced by shift-work and higher risk of cancer. In the first Nurses' Health Study cohort, involving 121,700 female nurses younger than 55 years at study entry, extended periods of rotating night work were significantly associated with a 36% increased risk of breast cancer, colorectal cancer (35%), and endometrial cancer (43%), independent of other known risk factors for each of these cancers [85–88]. A subsequent Nurses' Health Study cohort, involving 116,678 female nurses younger than 42 years at study entry, further confirmed the risk increase in breast cancer, with an even more important increase in relative risk (79%) [88]. In men, rotating shift-work was found to be significantly and independently associated with increased risk of prostate cancer, in two independent cohorts in Japan and Canada [89,90]. These human epidemiologic studies, and evidence from rodent studies for the carcinogenicity of light during the biological night, led a 24-members Working Group for the World Health Organization International Agency for Research on Cancer to conclude that shift-work, that leads to circadian disruption, was probably carcinogenic for humans (level of evidence 2A) [91].

Experimental data linking the molecular clock and the cell division cycle (reviewed in [92–97]) have raised the possibility that clock genes may play an important role in human cancer development and progression. Large population-based studies have documented a relatively high degree of polymorphism in human clock genes [98–103], and several of the germline clock genes variants have been shown to affect the phenotype of the subject [1,13]. In recent case-control studies, clock gene polymorphisms have been associated with a significant modification of the risk of non-Hodgkin lymphoma,

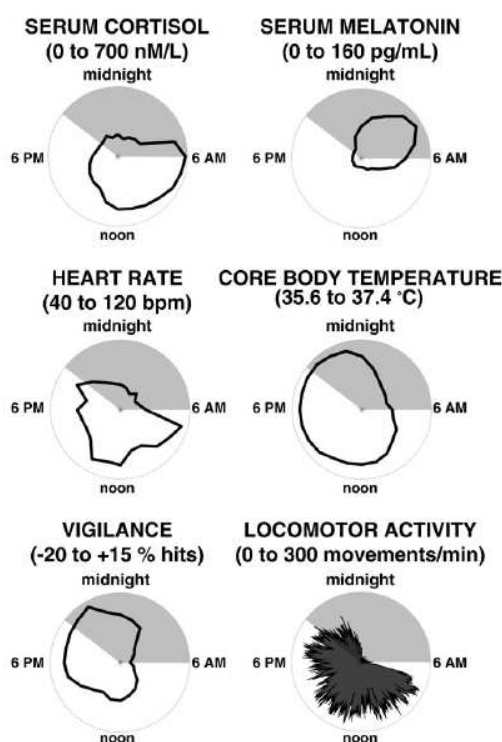


Fig. 1. Examples of six markers of circadian physiology in humans. The white part of the circle represents daylight (from 06h00 till 22h00), the grey part represents the night (from 22h00 till 06h00). For each rhythm, the scale indicated goes from the centre of the circle (lowest value) to the periphery or the circle (highest value). Serum cortisol (upper left panel), with low values in the night, peak in the early morning, and decreasing values through the day; serum melatonin (upper right panel), with high values at night and extremely low values through the day; heart rate (middle left panel), with higher values during active daytime, and lower values during nighttime rest; core-body temperature (middle right panel), whose decrease coincides with nighttime sleep; vigilance (bottom left panel), expressed as percentage of correct reaction to a stimulus, with lowest values at subjective night and better performance during daytime; locomotor activity (bottom right panel), with large difference between the daytime active span and the restful nighttime span.

Table 1

Published data on clock genes expression at mRNA and/or protein levels in human healthy tissues and/or cancers.

Tissue/tumor	No. of subjects/patients	Clock gene(s)	Gene product	REF
<i>Multiple time points</i>				
Oral mucosa	10	Transcriptome	mRNA	[67]
Mammary epithelium	5	Transcriptome	mRNA	[49]
Adipose tissue	17	Transcriptome	mRNA	[48]
Peripheral blood mononuclear cells	3	Per1, Per2, Per3, Dec1	mRNA	[38]
Peripheral blood mononuclear cells	10	Per2, Bmal1, Rev-erb- α	mRNA	[53]
Peripheral blood mononuclear cells	8	Per1, Per2, Per3, Clock, Bmal1, Cry1, Cry2, Dec1, Dec2, Tim	mRNA	[46]
Peripheral leukocytes	40	Clock, Bmal1, Per1, Per2, Per3, Cry1	mRNA	[64]
Oral mucosa	8	Clock, Tim, Per1, Cry1, Bmal1	mRNA	[37]
Skin	8	Clock, Tim, Per1, Cry1, Bmal1	mRNA	[37]
Bone marrow CD34+ cells	10	Per1, Per2, Cry1, Cry2, Bmal1, Rev-erb- α , Clock	mRNA	[55]
Peripheral leukocytes	6	Cry1, Per1, Per2	mRNA	[379]
Chronic lymphocytic leukemia cells	6	Cry1, Per1, Per2	mRNA	[379]
<i>Single time point</i>				
Epiphysis	68	Bmal1, Cry1, Per1, Clock	mRNA	[56]
Heart	52	Per1, Per2, Bmal1, Cry1	mRNA	[47]
Colon mucosa	25	Per1, Per2, Clock, Bmal1	mRNA and protein	[50]
Colorectal mucosa	169	Per1, Per2, Per3	Protein	[59]
Colorectal cancer	198	Per1, Per2, Per3	Protein	[59]
Colorectal mucosa	30	Per1, Per2, Clock	mRNA	[60]
Colorectal cancer	30	Per1, Per2, Clock	mRNA	[60]
Colorectal mucosa	30	Per1, Per2, Clock	mRNA	[61]
Colorectal cancer	30	Per1, Per2, Clock	mRNA	[61]
Lung	33	Per1	mRNA	[42]
Non-small cell lung cancer	33	Per1	mRNA	[42]
Lung	77	Per1	mRNA	[43]
Non-small cell lung cancer	77	Per1	mRNA	[43]
Breast	6	Per1	mRNA	[42]
Breast cancer	24	Per1	mRNA	[42]
Breast	55	Per1, Per2, Per3	Protein	[39]
Breast cancer	55	Per1, Per2, Per3	Protein	[39]
Breast	53	Per1, per2, Per3, Cry1, Cry2, Bmal1, Clock, Tim1, CK1 ϵ	Protein	[45]
Breast cancer	53	Per1, per2, Per3, Cry1, Cry2, Bmal1, Clock, Tim1, CK1 ϵ	Protein	[45]
Breast	58	Per1, Per2	mRNA	[65]
Breast cancer	58	Per1, Per2	mRNA	[65]
Pancreas	6	Per1, Dec1	mRNA	[62]
Pancreatic cancer	21	Per1, Dec1	mRNA	[62]
Endometrium	35	Per1	mRNA and protein	[57]
Endometrial cancer	35	Per1	mRNA and protein	[57]
Endometrium	35	Per1, per2, Per3, Cry1, Cry2, Bmal1, Clock, Tim1, CK1 ϵ	mRNA	[51]
Endometrial cancer	35	Per1, per2, Per3, Cry1, Cry2, Bmal1, Clock, Tim1, CK1 ϵ	mRNA	[51]
Endometrium	35	Per1, Per2, Cry1	Protein	[52]
Endometrial cancer	35	Per1, Per2, Cry1	Protein	[52]
Liver	46	Per1, per2, Per3, Cry1, Cry2, Bmal1, Clock, Tim1, CK1 ϵ	mRNA and protein	[58]
Hepatocellular carcinoma	46	Per1, per2, Per3, Cry1, Cry2, Bmal1, Clock, Tim1, CK1 ϵ	mRNA and protein	[58]
Ovary	11	Per1, per2, Per3, Cry1, Cry2, Bmal1, Clock, CK1 ϵ	mRNA	[54]
Ovarian cancer	83	Per1, per2, Per3, Cry1, Cry2, Bmal1, Clock, CK1 ϵ	mRNA	[54]
Prostate	152	Per1	mRNA	[44]
Prostate cancer	282	Per1	mRNA	[44]
Pleura	6	Transcriptome	mRNA	[66]
Mesothelioma	5	Transcriptome	mRNA	[66]
Chronic myeloid leukemia	35	Per1, Per2, Per3, Cry1, Cry2, Bmal1	mRNA	[322]
Acute myelogenous leukemia	21	Per2	mRNA	[41]
Chronic lymphocytic leukemia	116	Per1, Per2, Cry1	mRNA	[379]
Breast cancer	348	Npas2	mRNA	[378]

breast and prostate cancers [104–111] (Table 2). It is interesting to note that a given clock gene might have several polymorphisms, each of whom might be associated with higher or lower risk of a given cancer (e.g. NPAS2 and prostate cancer, with rs895521 and rs1369481 associated with lower risk, and rs17024326 associated with higher risk), and that a single polymorphism may be associated with decreased risk of more than one cancer (e.g. NPAS2 rs2305160 with lower risk of non-Hodgkin lymphoma, breast and prostate cancers) (Table 2). While the precise mechanisms linking clock genes single nucleotide polymorphisms (SNPs) and cancer risk remain unclear, the molecular clock is linked to hematopoietic pathways and estrogen- and androgen receptor pathways. These pathways are important in non-Hodgkin lymphomas, breast and prostate cancers, respectively [41,44,112–115].

In addition to its association with higher cancer incidence, circadian disruption has been shown to be associated with faster tumor growth in experimental models [116–118] and with shorter survival in clinical studies. Patients with metastatic breast cancer, whose diurnal salivary cortisol slope was abnormal (flat or inverted with night peak), had a 50% reduction in median survival as compared to patients with normal salivary cortisol pattern (morning surge and diurnal steep slope) [119]. The impact of circadian disruption on survival was confirmed in two separate studies involving a total of 322 patients with metastatic colorectal cancer, where the patients' circadian locomotor activity pattern was continuously recorded by a wrist-worn actigraph for 72 h [120,121]. Circadian disruption was found in about a quarter of the patients and was significantly associated with a 50% reduction in median survival, in both studies [92,120,121] (Fig. 3).

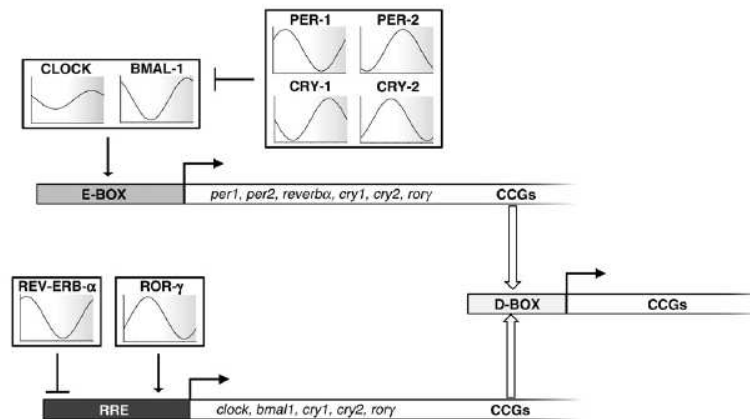


Fig. 2. Schematic representation of the ubiquitous transcription control of clock genes and clock-controlled genes through cyclic induction or inhibition of transcription by clock proteins, acting through specific promoters, E-boxes or ROR elements. Among the clock-controlled genes, other transcriptional factors, such as DBP, HLF, TEF or E4PB4, regulate the oscillatory transcription of clock-controlled genes through D-boxes. The curves show relative mRNA amount of main clock genes throughout the 24-h span. CCGs: clock-controlled genes. Adapted from [30,68].

Table 2

Published data regarding the significant associations between clock genes polymorphisms and cancer risk, either increased (OR>1) or decreased (OR<1).

Disease	Gene	Polymorphism	# Cases/# controls	Odds ratio (95% CL)	Reference
Prostate	NPAS2	rs895521	1308/1266	0.83 (0.70–0.97)	[104]
Prostate	NPAS2	rs1369481	1308/1266	0.81 (0.69–0.95)	[104]
Prostate	NPAS2	rs17024326	1308/1266	1.25 (1.07–1.47)	[104]
Prostate	ARNTL (BMAL1; MOP-3)	rs7950226	1308/1266	1.22 (1.02–1.46)	[104]
Prostate	CSNK1E	rs1534891	1308/1266	2.65 (1.16–5.95)	[104]
Prostate	PER3	rs1012477	873/1266 ^a	1.25 (1.03–1.52)	[104]
Prostate	CRY2	rs2292912	873/1266 ^a	0.82 (0.69–0.99)	[104]
Prostate	ARNTL (BMAL1; MOP-3)	rs7950226	873/1266 ^a	1.22 (1.00–1.49)	[104]
Prostate	NPAS2	rs17024926	873/1266 ^a	1.28 (1.07–1.53)	[104]
Prostate	NPAS2	rs1369481	873/1266 ^a	0.80 (0.67–0.96)	[104]
Prostate	CSNK1E	rs1534891	873/1266 ^a	3.09 (1.32–7.21)	[104]
Prostate	CRY1	rs12315175	873/1266 ^a	1.55 (1.01–2.39)	[104]
Prostate	PER2	rs7602358	873/1266 ^a	1.24 (1.03–1.50)	[104]
Prostate	CLOCK	rs11133373	435/1266 ^b	0.79 (0.63–0.99)	[104]
Prostate	NPAS2	rs895521	435/1266 ^b	0.79 (0.62–0.99)	[104]
Prostate	PER1	rs885747	435/1266 ^b	0.71 (0.51–0.99)	[104]
Prostate	PER1	rs2289591	435/1266 ^b	1.70 (1.08–2.66)	[104]
Prostate	NPAS2	rs2305160	187/242	0.5 (0.3–1.0)	[105]
Prostate	CRY2	rs1401417	187/242	1.7 (1.1–2.7)	[105]
Non hodgkin lymphoma	NPAS2	rs2305160	455/527	0.66 (0.51–0.85); p=0.001	[106]
Non hodgkin lymphoma	CRY2	rs11038689	455/527	2.34 (1.28–4.27); p=0.006	[107]
Non hodgkin lymphoma	CRY2	rs7123390	455/527	2.40 (1.39–4.13); p=0.002	[107]
Non hodgkin lymphoma	CRY2	rs1401417	455/527	2.97 (1.57–5.63); p=0.001	[107]
Breast	NPAS2	rs2305160	431/476	0.61 (0.46–0.81); p=0.001	[109]
Breast	PER3	AB047686	389/432	1.5 (0.9–2.5) ^c	[108]
Breast	PER3	AB047686	836/946	3.5 (1.1–11.5) ^d	[110]
Breast	CLOCK	rs3805151	836/946	1.7 (1.1–2.6) ^d	[110]
Breast	CLOCK	rs7698022	441/479	1.34 (1.02–1.76)	[111]
Breast	CLOCK	rs11133391	441/479	0.75 (0.56–0.99)	[111]
Breast	CLOCK	rs11932595	441/479	1.43 (1.07–1.91)	[111]
Breast	CLOCK	rs1048004	441/479	1.34 (1.02–1.76)	[111]
Breast	CLOCK	rs6850524	86/479 ^e	0.45 (0.27–0.76)	[111]
Breast	CLOCK	rs13102385	86/479 ^e	0.46 (0.27–0.76)	[111]
Breast	CLOCK	rs11932595	86/479 ^e	1.88 (1.06–3.31)	[111]
Breast	CLOCK	rs7698022	86/479 ^e	2.87 (1.25–6.59)	[111]
Breast	CLOCK	rs1801260	86/479 ^e	2.57 (1.14–5.82)	[111]
Breast	CLOCK	rs1048004	86/479 ^e	2.69 (1.18–6.13)	[111]

^a Subgroup of men with less aggressive tumor.

^b Subgroup of men with more aggressive tumor.

^c Significant association in the subgroup of premenopausal women (cases = 88; controls = 149); OR: 1.7 (1.0–3.0).

^d Significant association only in the subgroups with more than one parity or longer period of breast feeding (with the corresponding ORs in the table).

^e Subgroup of estrogen and progesterone receptors negative cases.

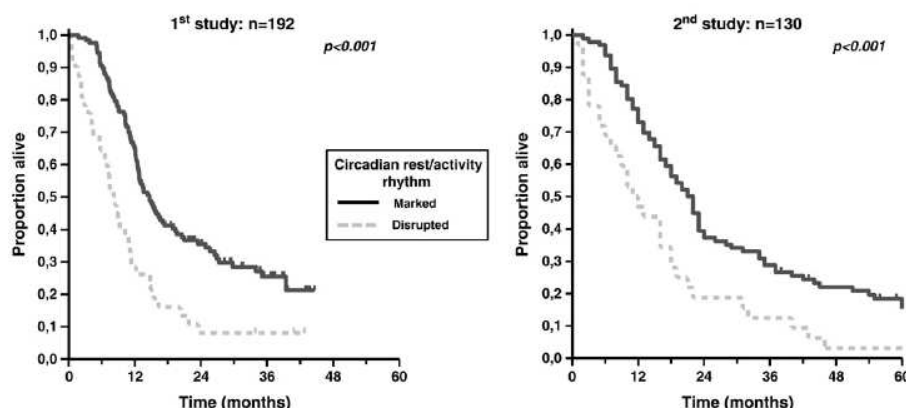


Fig. 3. Survival curves of patients with metastatic colorectal cancer according to the presence or absence of circadian disruption before chemotherapy start. The locomotor rhythm, recorded by a wrist-actigraph, was used as circadian biomarker. Circadian disruption was defined as being present in patients whose dichotomy index was in the lowest quartile (dashed grey line). The left panel shows data from 192 patients, receiving chronomodulated chemotherapy, as first or 2nd line treatment. The right panel shows data from 130 previously untreated patients receiving either chronomodulated or conventional chemotherapy. Redrawn with data from [120,121].

1.3. Cancer chronotherapy: methodological approaches

The time of cancer drug administration is rarely stipulated in study protocols or reported in publications of clinical trials [122]. As a result, dosing time varies both among and within patients, with most intravenous chemotherapy being given during daily working hours for the convenience of hospital staff [1]. For oral drugs given once daily, morning administration seems to be preferred [123–127]. The rationale for this dosing time is usually not given and patient compliance is not reported [128–133]. This lack of attention to the timing of therapy assumes that biological parameters are either constant throughout the 24-h, or that their variations are unpredictable and/or stochastic. Circadian-based chemotherapy (chronotherapy), on the other hand, attempts to rationalize the administration time for each drug in order to optimize its therapeutic index. The timing of therapy is then based on data on pertinent circadian rhythms and rodent and human chronotherapy studies.

The parameters commonly used to define the administration protocol for an infusional drug include dose administered per unit (usually body surface area or body weight), duration of infusion and frequency of administration. The delivery flow rate is usually constant (i.e. flat) along the duration, except for drugs that can cause serious allergic reactions, when starting with a low infusion rate and then increasing the rate is sometimes suggested. Timing of therapy can be taken into account with a constant infusion rate if the drug is given for less than 24 h. Then the time of starting and stopping the infusion is stipulated and reported. Chronomodulated delivery introduces another infusion parameter, the time of peak-flow rate. The administration pattern is not constant, but semi-sinusoidal, with an increasing flow rate, a peak delivery rate at a specified time, and a gradual symmetric decrease in flow rate. The chronomodulated delivery profile is particularly suitable for, but not restricted to, drugs with a short half-life where a relatively long duration of administration is preferred.

A constant-rate infusion over >24-h, or integral multiples of this span, does not take circadian biology into account. This delivery schedule has therefore been used as a control arm for studies of cancer chronotherapy for drugs whose pharmacologic properties permit long term infusion [1]. When the chemotherapy drug needs to be infused over a short time, chronotherapy studies can be done with a timed bolus [122] or a short infusion where the timing of administration start, peak and stop are stipulated [1].

In clinical practice the time of administration is ignored and varies along the daytime working hours. This conventional approach cannot be used as a control arm for testing the impact of circadian-based therapies, especially if the expected best time of administration takes place during the day. The dissimilarities between the conventional and the chronomodulated arm are not limited to the time of administration, but include differences in delivery profile, infusion duration and drug sequence (e.g. [134–136]). Therefore, the assessment of the clinical impact of the time of administration, and subsequently the interpretation of the results, become more complicated and problematic [137].

2. Circadian time-dependent therapeutic index

The therapeutic potential of circadian-based chemotherapy against cancer is mainly dependent upon the control that the molecular clock exerts on drug metabolism and multiple cellular processes (cell cycle, apoptosis, angiogenesis and DNA repair) that are important molecular determinants of cellular pharmacokinetics and pharmacodynamics of a cytotoxic/cytostatic drug [12,16,92–97,138–149]. In addition, the circadian timing system of the host can influence the replication and attrition rates of a tumor cells through the rhythmic variations in growth and/or death signals, conveyed by cytokines, inflammatory mediators, cortisol and catecholamines [150–169]. It is important to note that the optimal time for each cancer drug cannot be predicted from our current understanding of how circadian rhythms impact on their metabolism and activity. Detailed rodent studies, testing multiple administration times followed by clinical trials guided by these results are still required. It is important to emphasize a chronobiologic difference between experimental rodents and humans: while humans are diurnal, mice and rats are nocturnal. Therefore locomotor activity and feeding are mainly restricted to the dark phase in rodents. Therefore most physiological rhythms, and clock genes expression patterns display a ~12-h phase shift between rodents and humans [1,2,4,9,10,26,143,170,171]. This phase difference needs to be accounted for when experimental findings from rodents are translated into clinical trials.

The circadian timing system temporally controls the pharmacologic determinants of xenobiotic drugs both at the whole body and at the cellular levels via circadian rhythms in physiology and in molecular pathways (Fig. 4). This may result in circadian time-dependent difference in the therapeutic index. Rodent experimental models have demonstrated a clear pattern of chronotolerance for 40

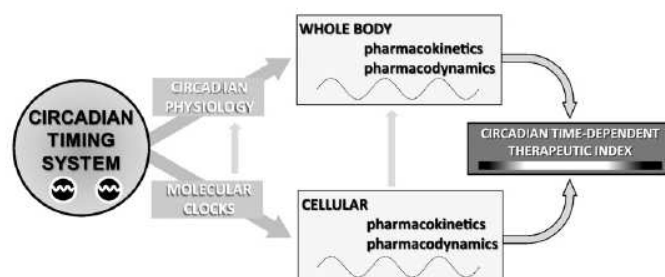


Fig. 4. The circadian timing system control of pharmacologic determinants of xenobiotic drugs that forms the basis for the circadian time-dependent therapeutic index of cancer therapy. This includes regulation of pharmacokinetics and pharmacodynamics at both whole-body and cellular levels.

cancer drugs including all the main pharmacologic classes of antineoplastic agents [1]. The mechanisms underlying the chronotoxicity patterns demonstrated in experimental rodents are multiple and specific to each drug, rather than to the class [1]. Below, we will provide an example of the mechanisms behind the circadian time-dependency of the therapeutic index of 5-fluorouracil (5-FU). 5-FU is an antimetabolite with broad activity against many types of solid tumors. It is widely used on several different schedules including bolus, and short- or protracted infusions. This prodrug, after undergoing intracellular activation, inhibits thymidylate synthase, thus disrupting DNA synthesis and repair, inducing lethal DNA damage, and altering RNA processing and function, thus affecting cell metabolism and viability [172].

2.1. Whole-body and cellular pharmacokinetics

The plasma concentration profile of 5-FU, administered continuously at a constant rate over at least 24 h, displays a circadian pattern with a trough at night [2,173]. A circadian rhythm in 5-FU plasma clearance was shown in patients receiving protracted low-dose constant-rate infusion of the drug for 2 weeks [1,173]. 5-FU catabolism by plasma dihydropyrimidine dehydrogenase activity, which displays a circadian pattern with the same phase, explained the 5-FU pharmacokinetic circadian profile [1,173]. Plasma dihydropyrimidine dehydrogenase activity was shown to be higher at night as compared to the day in two studies involving 7 patients with

gastrointestinal malignancies and 16 patients with nasopharyngeal carcinoma, respectively [174,175] (Fig. 5). However, high dose infusion of 5-FU at constant rate resulted in higher drug plasma concentration at night, since dihydropyrimidine dehydrogenase activity was saturated [2,173]. This difference in 5-FU concentrations rhythm according to the dose administered was further confirmed in a study involving 35 patients with esophageal squamous cell carcinoma receiving 5-FU continuous infusion [176].

At the cellular level 5-FU catabolism is carried out by dihydropyrimidine dehydrogenase, which mostly influences the intracellular pharmacokinetic profile of this antimetabolite [172]. The activity of this detoxifying enzyme in the cells of the oral mucosa of 8 healthy subjects was also higher at midnight than at 10h00 [177] (Fig. 5). Intracellular 5-FU catabolism, therefore, is accelerated at night, resulting in a lower exposure of intracellular targets to the active 5-FU metabolites at this circadian stage.

2.2. Whole-body and cellular pharmacodynamics

DNA synthesis in the main target tissues of 5-FU-induced toxicity (bone marrow, skin, and oral and rectal mucosa) is lowest during the night and highest during daytime [1,172,178–184] (Fig. 5). Therefore, at night, when the whole-body clearance of 5-FU is increased, the proportion of healthy cells potentially damaged by 5-FU is decreased. Whole-body pharmacodynamics of 5-FU, therefore, displays variation along the circadian time scale, with a synchronous phase between

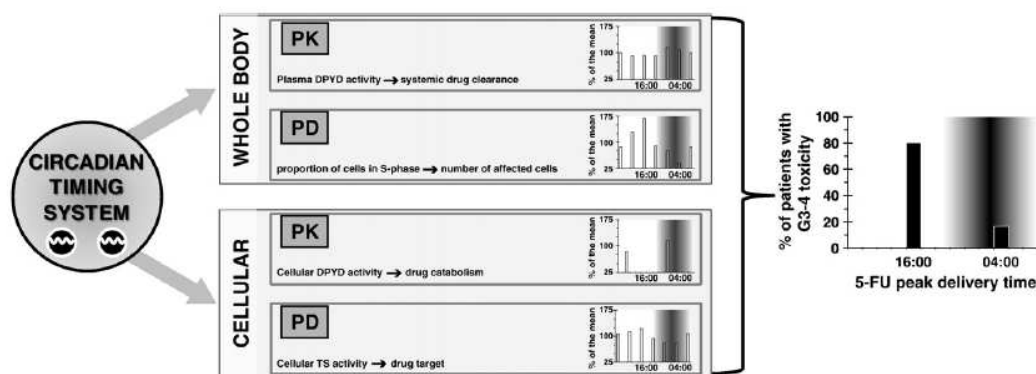


Fig. 5. Example of the conceptual frame depicted in Fig. 4, with human data for a commonly used chemotherapy drug, 5-FU. All four pharmacological determinants of 5-FU have been shown to display circadian variations along the 24-h span in humans, resulting in a better therapeutic index when 5-FU is administered during the night as compared to daytime administration. This has been confirmed by a clinical study that reported a ~5-fold greater rate of moderate or severe toxicity with 5-FU peak flow at 16h00 when compared to peak flow at 04h00. Adapted from [36,37,175,177].

different target tissues. The anabolic enzymes that generate the cytotoxic forms of 5-FU, (orotate phosphoribosyltransferase, uridine phosphorylase, and deoxythymidine kinase), have their highest activity during the dark (activity) span of rats or mice, when 5-FU is most toxic to healthy tissues [2].

The activity of the target enzyme of 5-FU has also been investigated at the cellular level in the oral mucosa cells of 6 healthy volunteers [178]. Thymidylate synthase activity showed a circadian rhythm, with a trough between midnight and 04h00 [178] (Fig. 5). At night, therefore, the molecular target of 5-FU is less active, resulting in a cellular chronopharmacodynamic pattern of this drug consistent with its lower cytotoxicity to the oral mucosa during the night.

The circadian profiles of whole-body and cellular chronopharmacokinetics and chronopharmacodynamics in humans would therefore predict a better tolerability of healthy tissues for a nightly administration of 5-FU. This hypothesis has been validated in several clinical studies (Fig. 4, cf *infra*, Section 3.1.2).

3. Cancer chronotherapy: clinical trials

Several early non-comparative trials tested the feasibility of chemotherapy, stipulating drug-delivery times along the 24-h [1,185,186]. These trials formed the basis for comparative trials, whose main endpoint were toxicity (phase I), response (phase II) or survival (phase III). Fig. 6 graphically depicts the methodological approaches used for the clinical testing of circadian-based chemotherapy in comparative trials. For intravenous therapy, the drug-delivery schedules include constant, bolus and chronomodulated patterns. Fixed-rate infusions can be either protracted (i.e., for over 24 h) or short (i.e., for less than 24 h), constituting a constant or bolus administration schedule, respectively. Chronomodulated profiles, instead, have time-varying infusional rates, as described above. For oral therapy, the time of a once/daily dose, or the relative daily dose fraction at each of the two equidistant dosing times can be specified. When assessing the chronotherapeutic relevance of a drug, the infusional route of administration has the disadvantage of requiring dedicated multichannel programmable pumps [1,187], whereas the oral route of administration has the drawback of inter-individual variability in absorption and compliance [2,133,188]. Table 2 lists the published comparative trials, in which at least one drug was administered on a chronotherapy schedule. The data in the table is classified based on the five possible methodological approaches schematized in Fig. 6. Trials where the dosing time was stipulated only in one arm and compared to “a conventional schedule” were excluded, because of the problems associated with this design discussed above [122,137]. Below we will discuss in more details one example for each of the five methodological comparative approaches (Table 3 and Fig. 6).

3.1. IV administration route

3.1.1. Chronomodulated versus constant

The human data supporting increased tolerability of 5-FU administered at night match the experimental evidence in rodents with the least toxicity associated with administration during the middle of the animal rest phase [1,2,189–191]. For the platinum complex oxaliplatin, however, best tolerability corresponds to the first half of the active phase in rodents [1,192]. One of the mechanisms involved in this chronotolerance pattern is the detoxification of oxaliplatin via reduced glutathione (GSH), whose liver concentration peaks during the second half of the dark span [1,193]. Corresponding data exist in humans, with a blood peak of GSH around noon [1,175]. As predicted by these rodent data, a randomized phase-I study including 23 patients with advanced cancers showed a better safety profile for a chronomodulated schedule of oxaliplatin with a peak delivery at 16h00 in comparison with a constant-rate infusion [194]. Based on the synergistic effects of the combination of oxaliplatin and 5-FU [195,196], further chronomodulated schedules were developed with this combination. A single-arm phase-II trial demonstrated adequate safety and

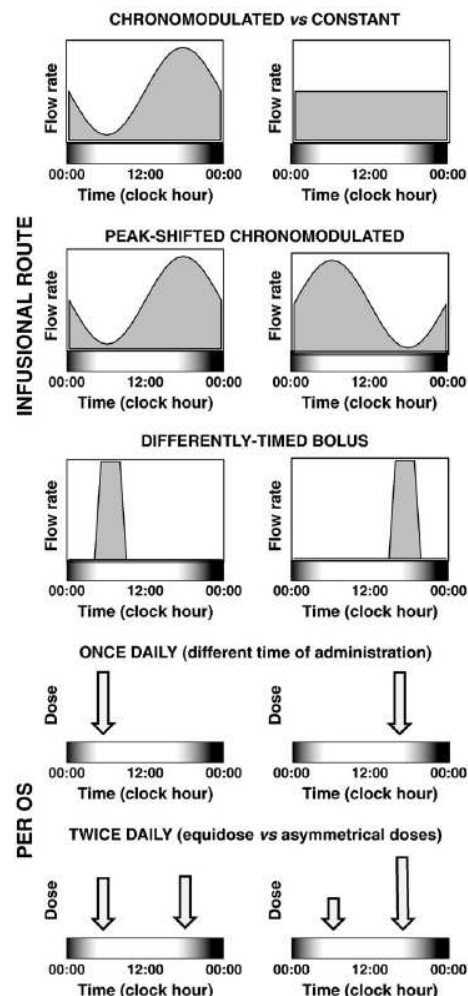


Fig. 6. Examples of chemotherapy administration profiles used in comparative trials that stipulate drug-delivery timing along the 24-h day. The upper three panels show drug-delivery profiles involving intravenous administration route, with comparison between a chronomodulated and a continuous delivery, between chronomodulated profile with different peak-flow times, and between bolus perfusions administered at different circadian times. The bottom two panels show two approaches of oral administration route of chemotherapy, for drugs given once daily, for which two different hours of administration are compared, or for drugs given twice a day, for which the comparison involves 50% of the daily dose given at either time points with unequal doses, with a greater fraction of the daily dose given at one time point and a lower fraction at the other time point. See references for each of these five approaches in Table 2.

unusually high efficacy for the combination of chronomodulated oxaliplatin (20 mg/m²/day, from 10h15 till 21h45, with a peak at 16h00) with folinic acid (300 mg/m²/day) and 5-FU (600 mg/m²/day, both from 22h15 till 09h45, with peak-flow rate at 04h00), given for 5 consecutive days every third week in patients with metastatic colorectal cancer [197]. This led to a proof of principle comparison of this chronomodulated schedule with an equidose administration of the

Table 3

List of comparative trials using circadian timing in cancer therapy, grouped based on to the methodological approach shown in Fig. 6. For phase-I trials, an evaluation of regimen activity (objective response, progression-free or overall survival) was not performed. IV: intravenous route. IHA: intra-hepatic artery administration route. NA: not applicable. NE: not evaluated. 5-FU: 5-fluorouracil.

Disease	# of patients	Trial type (phase)	Drug(s) tested	Association	Difference in toxicity/dose intensity	Difference in activity	Ref
<i>Chronomodulated vs constant</i>							
Metastatic colorectal	186	III	Oxaliplatin; 5-FU	No	Yes	Yes	[198]
Metastatic colorectal	92	III	Oxaliplatin; 5-FU	No	Yes	Yes	[199]
Metastatic colorectal	50	III	Floxuridine (IHA)	No	Yes	No	[463]
Metastatic colorectal	56	III	Floxuridine (IHA); 5-FU (IV)	No	Yes	No	[464]
Advanced cancers	23	I	Oxaliplatin	No	Yes	NA	[194]
Advanced cancers	36	I/II/III	Floxuridine	No	Yes	No	[465]
Advanced cancers	24	I/II	Carboplatin	No	No	No	[466]
Metastatic colorectal	48*	II	5-FU	Yes (oxaliplatin)	No	No	[467]
Metastatic colorectal	320	III	5-FU	Yes (mitomycin-C)	Yes §	No	[468]
Metastatic breast	60	II	5-FU	Yes (paclitaxel)	Yes	No	[469]
Advanced gastric	18	I	5-FU	No	Yes	NA	[470]
Advanced or metastatic pancreatic	107	I/II	5-FU; cisplatin	No	Yes	No	[471]
Metastatic gastrointestinal	113	I/II	5-FU	No	Yes	No	[472]
<i>Peak-shifted chronomodulated</i>							
Metastatic colorectal	114	I	Oxaliplatin; 5-FU	No	Yes	NA	[36]
Advanced lung	45	I	Carboplatin; 5-FU	No	Yes	NA	[36]
Metastatic breast	90	I	Vinorelbine	Yes (5-FU)	Yes	NA	[209]
Metastatic colorectal	199	I	Irinotecan	Yes (oxaliplatin and 5-FU)	Yes	NA	[208]
Advanced cancers	14	I	5-FU	No	Yes	NA	[473]
Metastatic gastrointestinal	113	I/II	5-FU	No	No	No	[472]
<i>Differently-timed bolus</i>							
Ovary	31	I	Doxorubicin; cisplatin	No	Yes	NE	[186,217]
Ovary; bladder	23	III	Doxorubicin; cisplatin	No	Yes	No	[220]
Ovary	31	II	Tetrahydropyran-yl-doxorubicin; cisplatin	No	Yes	Yes	[221]
Advanced lung	124	III	Etoposide	Yes (cisplatin)	Yes	No	[474]
Ovary	7	I	Carboplatin	No	Yes	NA	[475]
Metastatic cancers	34	I	Etoposide	Yes (cisplatin)	Yes	NA	[476]
<i>Oral: once daily (different time of administration)</i>							
Metastatic renal cell	107	I/II	Sunitinib	No	No	NE	[132]
Advanced gastrointestinal stromal	60	I/II	Sunitinib	No	No	No	[223]
Acute lymphocytic leukemia	118	Retrospective	6-mercaptopurine; methotrexate	No	NA	Yes	[477,478]
<i>Oral: twice daily (equidose vs asymmetrical dose)</i>							
Advanced colorectal	141	II/III	Capecitabine	Yes (oxaliplatin)	No	No	[247]

NA: not applicable.
NE: not evaluated.

same drugs at a continuous constant-rate infusion over 120 h (5 days) every third week, in two separate international, randomized, controlled phase-III trials [198,199]. The first trial involved 92 chemo-naïve patients, and demonstrated significantly better tolerability (less toxicity despite higher dose intensity) and higher antitumor effectiveness (greater objective response rate and longer progression-free and overall survival) for the chronomodulated schedule as compared with the fixed-rate, flat infusion [199]. However, because of the potential chemical interaction between 5-FU and oxaliplatin, when administered concomitantly through the same catheter, this trial was stopped prematurely [199]. The subsequent trial compared the same test and control schedules, using a dedicated double-lumen implanted venous side port with separate channels [198]. This trial was interrupted prematurely based on the recommendation of an international advisory committee, because of better results on the chronotherapy arm after enrolment of 186 chemo-naïve patients. Not only was there a significant difference in the main clinical endpoint (objective response rate) favoring the chronomodulated schedule (51% versus 29%, $p=0.003$), but the rate of severe toxicities was up to 5-fold less in the patients on chronotherapy [198]. The increased efficacy with chronomodulated delivery was associated with a significant reduction in severe toxicities, a lower rate of hospital admissions for toxicity (10% versus 31%, $p=0.001$) and a lower proportion of patients

withdrawing due to toxicity (28% versus 51%, $p=0.002$) [198]. Thus, grade 3 or 4 oral stomatitis was experienced by 14% of the patients receiving chronomodulated infusion and by 76% of the patients with fixed-rate delivery ($p=0.0001$), and the rate of peripheral sensory neuropathy was almost halved with chronotherapy (16% versus 31%, $p=0.01$) [198]. The frequencies per course of severe diarrhea, palmo-plantar erythrodysesthesia (hand-foot syndrome) and leuco- and neutropenia were significantly greater for constant-rate chemotherapy ($p<0.05$), despite a 40% higher median dose of 5-FU per course with chronotherapy (3500 mg/m² versus 2500 mg/m²) [198]. Furthermore, more patients on the chronotherapy arm became eligible for subsequent surgery of down-sized metastases that were initially considered unresectable (25% versus 18%). While median progression-free survival was longer in the chronotherapy arm (9.8 months versus 7.9 months), this difference was not statistically significant ($p=0.20$) [198], but the trial was not powered for this endpoint [200]. Overall survival did not significantly differ between the two arms, but 22% of the patients on the constant-rate infusion arm subsequently crossed over to chronotherapy following treatment failure, potentially masking a survival advantage [198]. A concern has been raised about the choice of the control arm, i.e. a continuous and concomitant infusion of the drugs, because of intrinsic pharmacologic differences as compared with the intermittent, sequential

chronomodulated delivery [137,201]. The above work led to the largest international randomized trial to date to study the value of chronomodulated therapy for cancer [136] (Fig. 7).

The EORTC (European Organization for Research and Treatment of Cancer) Chronotherapy Group randomized 564 previously untreated metastatic colorectal cancer patients to receive equidosed standard (2 h-infusion of oxaliplatin and folinic acid, and flat infusion of 5-FU, FOLFOX2, [202]) therapy with 5-FU/folinic acid and Oxaliplatin or a chronomodulated infusion of the same drugs with peak Oxaliplatin delivery at 16h00 and 5-FU/folinic acid at 04h00 (chronoFLO4, [203]) [136]. While overall survival was the same in the standard and chronotherapy arms, there were significant gender differences in survival. In 338 male patients, the risk of death was decreased by 25% with chronotherapy versus standard therapy with median survival times of 21.4 and 18.3 months respectively ($p=0.0183$) [136]. However, in 226 female patients, the risk of death with chronotherapy was increased by 38% ($P=0.0269$) compared with standard therapy with median survival times of 16.3 and 19.1 months respectively [136]. Gender differences in survival were not seen on the standard therapy arm where timing of therapy was not stipulated (Fig. 8). Thus consistent timing of therapy amplified gender differences in survival (Fig. 8). These findings have been confirmed in the first meta-analysis of chronotherapy trials [204] (cf *infra*, Section 4.1). This novel connection between gender differences in outcome and treatment timing warrants attention in future cancer trials and should be considered early in the drug development process.

3.1.2. Peak-shifted chronomodulated

To study the possible effect of intermittent and sequential delivery on the therapeutic index of the oxaliplatin/folinic acid/5-FU combination, a comparison was performed between variable-rate chronomodulated infusions of these agents, differing only in the timing of peak delivery of the drugs. Two follow-up single-arm studies had shown a potential benefit from intensification of the chronomodulated delivery schedule for this combination by shortening the treatment duration to 4 days on a biweekly schedule [203,205]. Based on these data, the daily doses tested in this study were 25 mg/m²/day for oxaliplatin, 300 mg/m²/day for folinic acid and 800 mg/m²/day for 5-FU. Each drug was administered with a variable-rate semi-sinusoidal infusion profile lasting 11.5 h, with concomitant 5-FU and folinic acid in sequence with oxaliplatin [36]. The timing of peak delivery rates of the drugs was the only difference between the 8 treatment arms (staggered by 3 h), while the phase between

oxaliplatin and 5-FU/folinic acid was kept constant at 12 h [36]. In this phase-I trial, involving 114 patients with metastatic colorectal cancer [36], the primary endpoint was the incidence of severe toxicities after two courses of chronotherapy, with the hypothesis that the “reference” schedule (oxaliplatin peaking at 16h00 and 5-FU/folinic acid peaking at 04h00) would be least toxic, and the opposite one (respective peaks shifted by 12 h) would be the most toxic one. Indeed, 17% of the patients receiving chronotherapy with the reference schedule experienced at least one grade 3 or 4 toxicity during the first two courses of chemotherapy, whereas this rose to 80% on the opposite schedule, with intermediate rates in the other treatment arms [36]. The incidence of severe toxicity in the reference- and opposite schedule was 41% and 100% respectively after four courses of therapy [36]. The study identified the respective optimal times for peak delivery of chronomodulated 5-FU, folinic acid and oxaliplatin, with their 90% confidence limits after 2 and 4 courses. Interestingly, the optimal time was located at 04h00 over the initial 2 courses but at 06h20 over the subsequent 4 courses, suggesting that treatment itself could shift optimal timing in subsequent courses. Antitumor efficacy was also superior on the reference schedule, with an objective response rate of 30% versus 13% in the opposite schedule [36]. The reduced toxicity associated with chronomodulated 5-FU-folinic acid with a peak at 04h00 and chronomodulated platinum complex (carboplatin, whose preclinical chronotolerance profile matches that of oxaliplatin [1,206,207]) with a peak at 16h00 was confirmed in a randomized phase I trial involving 45 patients with advanced non-small cell lung cancer [36]. The study compared three chronotherapy schedules, with the same doses and intervals between drugs, yet with peak-flow rates staggered by 8 h. These two trials are the only ones involving combination regimens in which the respective phases between peak delivery rates of the drugs remained unchanged between treatment arms (i.e., fixed at 12 h).

In two other phase-I trials, the peak administration time of a single drug (Irinotecan or Vinorelbine) within the combination regimen was modified, while keeping the other drugs with the same chronomodulated profile and circadian timing. As a result, differences between tested schedules characterized not only the circadian timing of the drug of interest, but also the intervals between the drugs in the tested chronomodulated schedules [1,208,209]. Therefore, other pharmacological parameters may have been present, potentially obscuring the circadian dosing timing component [1]. Indeed, the trials primary endpoints were not met, even though toxicity rates varied largely among groups (irinotecan-induced diarrhea ranged from 34 to 52%)

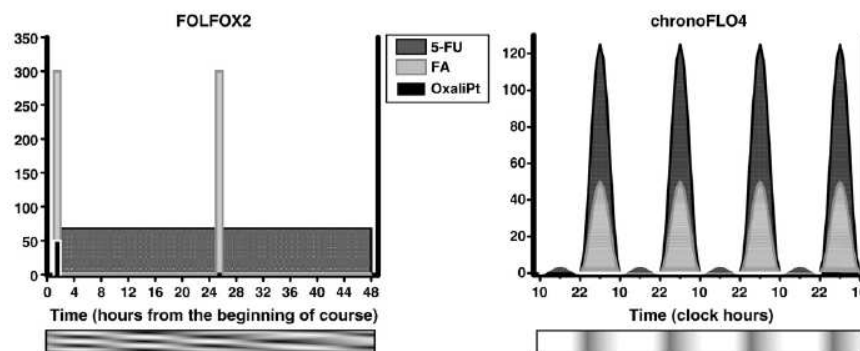


Fig. 7. Treatment schedules combining Oxaliplatin (OxaliPt, 100 mg/m²/course), 5-fluorouracil (5-FU, 3000 mg/m²/course) and folinic acid (FA, 1200 mg/m²/course) administered either as a conventional infusion over 2 days, with no time stipulation (FOLFOX2, left panel), or as a chronomodulated infusion during 4 days (chronoFLO4, right panel), given every second week. The ordinate represents drug-delivery rate (expressed as mg/m²/h), and the abscissa represents time, either from the beginning of infusion, with no relation to the clock hour (no time stipulation) for FOLFOX2, or as clock hour, for chronoFLO4. Adapted from [136].

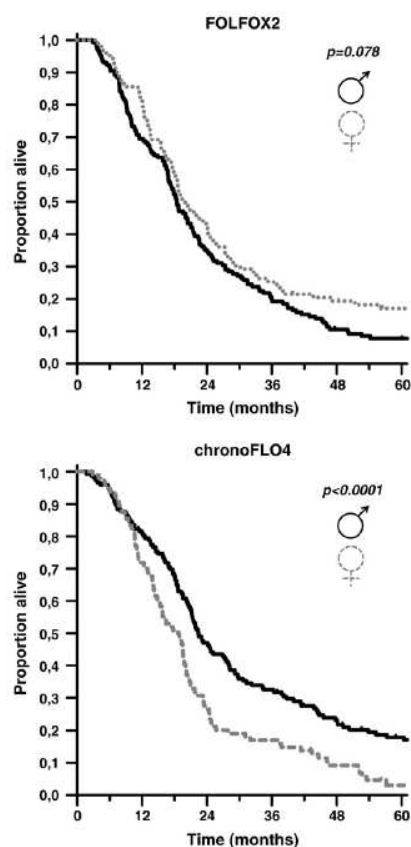


Fig. 8. Kaplan-Meier survival curves according to gender in patients receiving either the conventional schedule FOLFOX2 (upper panel) or the chronomodulated schedule chronoFLO4 (bottom panel). Redrawn from original data [136].

[1,208,209]. However, vinorelbine-induced leucopenia was the only circadian chronotolerance profile that was validated [209]. It may therefore be important to maintain a constant interval between drugs within a regimen in this kind of trial, to minimize confounding factors.

It is remarkable that the toxicity rate of the combination of oxaliplatin and folinic acid-modulated 5-FU is similar when these drugs are administered as a continuous flat rate infusion as when they are administered with a chronomodulated delivery opposite to the validated optimal chronomodulated schedule [36,198]. Furthermore, mathematical models that take into account the circadian molecular clock control of cell proliferation of healthy tissues have validated the empirical finding that the constant-rate infusion is as toxic as the "worst" possible chronomodulated schedule [210].

3.1.3. Differently-timed bolus

Cisplatin is often given in combination with doxorubicin. Cisplatin, displayed a preclinical chronotolerance pattern similar to that of oxaliplatin and carboplatin, with the lowest toxicity with administration during the first half of the rodent active span [1,206,211]. However, the DNA intercalator and Topoisomerase II- α inhibitor doxorubicin (adriamycin), was shown to be less toxic when

administered during the rodent rest span [1,212,213]. This chronotolerance profile did not seem to be related to the daily variation of pharmacokinetics of adriamycin [214], but was shared with other anthracyclines, including theprubicin [1,215]. The mechanism behind the chronotolerance of anthracyclines may involve both common and complex detoxification pathways and the multiple target mechanisms of their cytotoxic activity. Preclinical evidence of chronotoxicity and chronoefficacy profiles of doxorubicin and cisplatin combinations further demonstrated that the best therapeutic index (least toxicity and highest tumor response, complete remission and cure rate) of this combination is achieved when both agents are administered at the respective best tolerated times [216]. A clinical trial with a crossover design, showed a better therapeutic index for this combination when adriamycin was administered shortly before awakening and cisplatin in the evening [1,217,218]. These results led to a randomized trial involving 31 women with previously untreated advanced ovarian cancer [217]. This was the first trial to report an exploitable advantage of circadian-based chemotherapy without chronomodulated infusion. In this trial, treatment timing was the variable tested, but the starting dose (60 mg/m² for both adriamycin and cisplatin), the order of drug administration and the time span between the two drugs were kept constant. The times of drug administration were 06h00 and 18h00 [217]. Morning administration of adriamycin and evening administration of cisplatin were associated with less toxicity when compared to the inverse sequence, in terms of rates of dose reductions and treatment delays per patient (40% versus 81%, respectively; $p < 0.05$), as well as hematologic toxicity (53% versus 88%, respectively; $p < 0.05$) [217]. A subsequent update and extension of this cohort reported a better 5-year survival rate in patients treated with the least toxic schedule (morning adriamycin and evening cisplatin) as compared to the opposite one (44% versus 11%) [186,219]. The better tolerability of morning adriamycin and evening cisplatin was confirmed by another phase-II trial involving 23 patients with ovarian or bladder cancers [220] and a randomized phase-II trial of theprubicin (4'-O-tetrahydropyranyl-doxorubicin) and cisplatin [221]. The advantage of morning theprubicin and evening cisplatin in 31 women with advanced ovarian carcinoma resulted from lower hematological and renal toxicity, and higher dose intensity and response rate [221]. A randomized phase-III trial compared the circadian-timed combination of doxorubicin (at 06h00) and cisplatin (at 18h00) with a standard (i.e., conventional, unrestricted timing) equidose schedule in 342 women with advanced, metastatic or recurrent endometrial cancer. The study did not demonstrate any significant survival benefit for the circadian-based chemotherapy, despite less severe leuko-neutropenia rate and slightly higher dose intensities [222]. However, as previously highlighted [122], the "conventional" administration with no time specification does not represent a relevant control arm.

3.2. PO administration route

3.2.1. Once daily (different dosing times)

Despite the fact that oral drugs with once-daily administration schedule represent the easiest model for studying circadian time-based scheduling, in oncology, only sunitinib has been the subject of such prospective comparison. In both studies, treatment timing was a secondary endpoint [132,223]. Sunitinib is a novel oral, selective multi-targeted tyrosine kinase inhibitor with antiproliferative effects against cancer cells and anti-angiogenic properties [224]. Sunitinib was recently approved by regulatory agencies for the first-line treatment of patients with advanced renal cell carcinoma and for the treatment of patients with gastrointestinal stromal tumors after disease progression or intolerance to imatinib therapy [225]. Although not always clearly specified, its tolerability in clinical trials has been evaluated mostly with morning administration [132]. Morning and evening administration was compared in a phase-II study where sunitinib was administered on a continuous once-daily dosing

regimen to 107 patients with cytokine-refractory metastatic renal cancer, with the hypothesis that evening administration would be associated with less toxicity [132]. This open-label trial was powered to evaluate objective response in the whole population, with physician-scored toxicity and patient-reported quality of life in the two dosing regimens as secondary endpoints [132]. Thus, no comparison was scheduled in terms of efficacy between morning and evening administration, and no statistical comparison of treatment arms was performed in terms of tolerability. There was no stated rationale for the two dosing times on this study. The median daily dose given was the planned 37.5 mg in both arms, given continuously [132]. The safety profile was similar in the two arms, overall, as reflected in the rates of discontinuations of therapy due to toxicity, dose interruptions, and dose escalations [132]. Nonetheless, the authors pointed out that patients receiving sunitinib in the morning appeared to have slightly lower rates of dose reduction (39% versus 47%). Moreover, severe fatigue and grade-3 diarrhea and neutropenia seemed less frequent in patients taking sunitinib in the morning (9% versus 23%, 6% versus 17%, 4% versus 13%, respectively) [132]. Health-related quality of life did not seem to change according to the dosing time of sunitinib [132]. Comparable results were reported for Sunitinib given on the same continuous schedule in 60 patients with imatinib-resistant/intolerant gastrointestinal stromal tumor [223]. While overall safety and efficacy was comparable between the morning and evening administration, grade 3 fatigue and asthenia were less frequent in patients taking sunitinib in the morning versus in the evening (3% versus 10%, and 7% versus 13%, respectively) [223]. A reduction in fatigue may be clinically meaningful since this symptom is associated with circadian disruption in cancer patients [11,120,121,226–234].

The maximum plasma concentration of sunitinib is achieved within 6–12 h [225,235]. Furthermore, an interpatient variability of 40% in total oral clearance has been reported [225,235], and confirmed by pharmacokinetic studies [223]. This large variability in pharmacokinetics might have masked a possible circadian time-dependent difference in tolerability. Moreover, the relatively long terminal half-life of sunitinib (40–60 h) [225,235], might render this continuous daily dosing schedule less dependent on the circadian time of administration.

Another study, contrasting morning and evening administration of the higher standard dose of Sunitinib (50 mg) given for 4 weeks with a 2 week break between cycles is ongoing (Bjarnason G., personal communication). Detailed imaging methods (dynamic contrast-enhanced ultrasound (DCE-US), DCE-magnetic resonance imaging and DCE-computed tomography) are used as surrogate markers of early anti-angiogenic activity during the first course of therapy. The rationale for this study is based on studies in mice with implanted tumors that documented a circadian rhythm in vascular endothelial growth factor (VEGF) mRNA and VEGF protein in tumor and plasma [236,237]. Per2 was found to down-regulate the expression of hypoxia-inducible factor 1 α (HIF-1 α)/aryl hydrocarbon receptor nuclear translocator (ARNT) induced VEGF promoter activity, with the rhythm in Per2 in antiphase with the VEGF rhythm [236,237]. Several anti-angiogenic drugs were more potent when injected at the time of day of the VEGF peak in tumor versus at the time of VEGF trough [236,237]. Since the tumor VEGF mRNA rhythm is in antiphase with the Per2 rhythm, it was hypothesized that the time of peak VEGF mRNA expression, and therefore the best time for anti-angiogenic therapy in human tumors, would be at night. This is based on three human studies showing that both Per1 and Per2 peak early in the day [37,38,238].

3.2.2. Twice daily (equal versus unequal daily dose fractionation)

Capecitabine is an oral fluoropyrimidine prodrug, which is converted by thymidine phosphorylase to 5-FU, that then is further metabolized and exerts its cytotoxic properties by inhibiting the activity of thymidylate synthetase, which provides the unique *de novo*

source of thymidylate [172,239]. Capecitabine is most commonly given in two equivalent daily doses, one in the morning and one in the evening [239,240]. The rationale behind studies giving a higher evening dose of capecitabine is based on the aforementioned data concerning 5-FU (Fig. 4). However, specific data on different asymmetric doses of capecitabine are lacking in rodents. Another 5-FU oral prodrug, tegafur, given in combination with the DPYD inhibitor uracil (UFT) and folinic acid was studied in 21 patients. A pharmacokinetic analysis found a higher exposure to the drug after a morning dose as compared to afternoon or evening dosing [241], in agreement with the data on intravenous 5-FU [173].

A chronomodulated schedule of capecitabine, with three doses per day, with half of the daily dose given in the evening, has been studied in single-arm trials either as monotherapy or in combination with oxaliplatin [242–244]. The safety and efficacy of this chronomodulated oral therapy with capecitabine was very good, but no conclusions could be drawn about the clinical value of this scheduling since there was no comparator arm.

In a single-arm phase-II trial involving 71 previously treated patients with metastatic colorectal cancer, capecitabine was administered with two daily asymmetrical doses (20% of the dose in the morning and 80% of the dose in the evening), in combination with oxaliplatin [245]. The safety (grade 3–4 toxicity rate) and efficacy (response rate, overall and progression-free survival) of this regimen was comparable to results obtained by the same group, in the same clinical setting (70 patients receiving second line treatment for metastatic colorectal cancer progressing after irinotecan/5-FU association), with a conventional schedule of capecitabine (50% of the dose in the morning and in the evening), in combination with oxaliplatin [246].

A randomized phase-II trial in 139 patients with chemo-naïve metastatic colorectal cancer compared conventional dosing of capecitabine (1000 mg/m² in the morning and in the evening) to chronomodulated administration, with 20% of the dose (400 mg/m²) given between 07h00 and 09h00, and 80% of the dose (1600 mg/m²) given between 18h00 and 20h00 [247]. Capecitabine was given continuously from the evening of day 1 to the morning of day 15 every third week, in combination with oxaliplatin (130 mg/m² IV, given between 13h00 and 15h00 on day 1). The authors hypothesized that this chronomodulation would result in a 20% reduction in the rate of grade 2 or higher toxicity. This primary endpoint was not met, with a rate of grade ≥ 2 toxicity of 85% in the chronomodulated arm and of 90% in the conventional arm ($p = 0.47$), with a comparable dose intensity of the two drugs in the two treatment arms [247]. Anemia and neutropenia seemed slightly more frequent in the conventional arm, whereas thrombocytopenia seemed a little more frequent in the chronomodulated arm [247]. Furthermore, response rate, progression-free survival and overall survival were similar in the two arms [247]. Thus, this chronomodulated schedule does not seem to improve the therapeutic index for capecitabine when given in combination with oxaliplatin [247]. The authors comment on other biological functions that influence oral drug availability and exhibit circadian variation [2,188] that were not accounted for, and the complex and largely unknown chronopharmacokinetics of capecitabine [247]. Indeed, five out of 21 patients receiving UFT/folinic acid displayed an opposite chronopharmacokinetic pattern to the one described earlier [241], with higher drug exposure in the evening than in the morning, highlighting the importance of inter-individual differences in circadian determinants, and their potential masking role [241]. Moreover, 5-FU concentration after an oral dose of capecitabine has been reported to peak after ~ 2 h [240]. Early evening (18h00–20h00) administration therefore leads to peak drug concentration much earlier than the 04h00 optimal time documented for 5FU [247]. It is possible that an adaptive-release formulation of capecitabine could improve its therapeutic index by optimizing the timing of the circadian exposure peak during the night rather than in late evening [1,187,248–253]. Such modified drug release has been used for

prednisone chronotherapy in patients with rheumatoid arthritis [254]. Overall, the chronotherapy trials using the oral route described have failed to demonstrate a clear time-dependency of the therapeutic index [132,223,247]. Possible explanations include; inter- and intra-individual variations in circadian absorption mechanisms [2,188,253], heterogeneity in patient compliance [133], and the fact that night time administration has not been studied. Furthermore, detailed around-the-clock preclinical chronopharmacologic studies have not guided the design of the clinical trials. This emphasizes the need for dedicated oral drug developmental processes that address chronobiological issues in preclinical studies, prior to proceeding to clinical trials. These preclinical studies should ideally identify the main chronopharmacological determinants for the drug under study, the role of the molecular circadian clock on pertinent cellular processes related to the drugs mechanism of action in healthy tissues, as well as the effects of the drug on the host circadian timing system.

4. Cancer chronotherapy: challenges and opportunities

4.1. Inter-individual and gender differences

There are many common germline polymorphisms in human clock genes, with no clearly associated phenotypes [98–103]. Inter-individual variation have been reported for many other circadian parameters, without an overt pathologic phenotype. For example, each human subject undergoes continuous cycles of alternating wakefulness and sleep, but the pattern of this rhythmic behavior is dependent on several factors, including genetic background, social and environmental cues, and other circadian rhythms [6,255–260]. This results in large inter-individual differences in the organization of human behavior within the 24-h day that impact on the preferred timing of sleep and wakefulness. Using a simple questionnaire (Munich ChronoType Questionnaire), a variable distribution of mid-sleep time and average sleep duration has been reported in more than 65,000 cancer-free human volunteers [261,262]. In particular, the mid-sleep time on non-working days (work-driven social schedules can drastically change sleep timing [261,262]) has been chosen as the marker that quantitatively defines the phase of the subject, or his/her chronotype [261,262]. Although the majority of individual chronotypes cluster around a mean phase (mid-sleep time on non-working days), the difference in hours between the extreme chronotypes spans over three quarters of the day [261,262]. Moreover, cultured fibroblasts derived from skin biopsies of cancer-free human subjects display a circadian oscillation of clock gene transcription, with period length that varies widely among individuals [263,264]. As has been shown for the different chronotypes, where the majority of period lengths averaged around 24.5 h, a maximum difference of 4 h was observed [263]. Furthermore, the amplitude and phase-shifting properties of the circadian oscillator could be different even between individuals with a similar fibroblast period length, [264], suggesting a possible inter-individual variability in the strength of the input signals to the circadian oscillators [264]. Given the tight link between core-body temperature circadian rhythm and sleep/wake cycles [265], and given the synchronizing effect of temperature oscillation on peripheral molecular clocks [266], it is possible that people with extreme phenotypes would display different entrainment of the peripheral clocks. The potential benefits associated with chronotherapy could clearly be increased if the timing of therapy could be individualized based on relevant circadian biomarker rhythms assessed in each patient.

More recently several data have found significant gender differences in the circadian system. This has major implications for chronotherapy in the clinic as was documented in the study discussed above where the benefit from chronotherapy with 5FU and oxaliplatin was not only restricted to males, but females did better on the standard arm than the chronotherapy arm [136].

A recent study on global gene expression in human oral mucosa has found significant gender differences in rhythmic gene expression [67]. There were over 2000 rhythmic transcripts in males and females but only several hundreds of these rhythmic transcripts were common to males and females and peaked at a similar time of day. Hundreds of rhythmic gene products involved in the signalling pathways currently targeted for cancer therapy had a rhythm and displayed significant gender differences [67]. This is consistent with other studies looking at gene expression in male and female mice at one time point. In the largest study, 169 female and 165 male mice were sacrificed at one time point and microarray analysis (23,574 transcripts) performed on samples from liver, adipose tissue, whole brain and muscle [267]. At the $p < 0.01$ significance level, 9250, 11,336, 4083, and 612 genes demonstrated gender differences in expression in liver, adipose, muscle, and brain, respectively. Furthermore, the tumor molecular phenotype has been reported to differ according to the gender of the host in which it arises, with important therapeutic implications [268–272].

The mechanisms responsible for gender differences in gene expression have not been fully characterized and the reason for their evolution in multiple species remains speculative [267,273]. Gonadal hormones contribute both directly and indirectly [274,275] and gender and tissue-specific transcription factor binding sites have been described [275–280].

Gender differences have been reported for many aspects of importance in colorectal cancer. Several large-scale studies have indicated that women have a substantially lower adenoma detection rate than men. Therefore adenomas are a less robust marker of colorectal cancer risk in women than in men. This has major implication for colorectal cancer screening [281–283].

A polymorphism in methylenetetrahydrofolate reductase has been shown to have a role as a prognostic marker in female patients with metastatic colon cancer treated with 5-FU based chemotherapy [284,285]. In both studies, survival was higher for C/C female patients than for A/C female patients. Another study documented a role for functional EGFR polymorphisms as independent prognostic markers in metastatic colon cancer, with the two variants having opposite prognostic implications based on gender [286].

There are well documented gender differences in drug metabolism that have major implications for the toxicity and activity of common cancer drugs [287]. As an example, hepatic p-glycoprotein is 2.4-fold lower in females than males [288]. This will reduce vinca alkaloids, doxorubicin, etoposide, and docetaxel elimination with a greater risk for myelosuppression and gastrointestinal toxicity in females as has been documented in lung cancer trials [289]. As a result, females experience greater drug toxicity with p-glycoprotein substrate drugs but can also have better responses due to a more prolonged drug exposure.

Significant gender differences in toxicity have also been documented for 5-FU based therapy for colorectal cancer, with less predictable 5-FU related toxicity in females [290,291]. Females display a lower average clearance of 5-FU and a less pronounced amplitude of the circadian rhythm in 5-FU clearance as compared to males [292]. The phase, however, was similar in males and females. The lower 5-FU clearance in females could explain the nearly doubled rate of severe toxicity experienced by females treated with chronomodulated 5-FU/folinic and oxaliplatin across the 8 tested times of peak drug-delivery rate as discussed above [36]. The pattern of toxicity differed according to sex, with a clearly identifiable optimal time for males, but a poorly defined and dampened profile in females, with the best peak time predicted to be 6 h later than in males [36]. The unmasking effect of gender on the inter-individual differences in chronomodulated chemotherapy efficacy has been recently confirmed in the first meta-analysis of chronotherapeutics [293]. Individual data from 842 patients with metastatic colorectal cancer enrolled in three randomized phase-III trials [136,198,294] comparing first-line

chronomodulated 5-FU, folinic acid and oxaliplatin versus conventional delivery of the same drugs confirmed that chronotherapy resulted in significantly better outcomes in males than in females, in terms of objective response rate, secondary resectability of metastases, time to progression and overall survival [293].

Studies looking at the timing of radiotherapy (RT) do also point to gender differences in radiation-induced toxicity. Based on the demonstration of a circadian rhythm in the human oral mucosa cell cycle, with most cells in the G1 phase in the morning and M phase at night [37,178], it was hypothesized that morning RT would lead to less oral mucositis than afternoon RT. A total of 216 patients (80% males) with head and neck cancer were randomized to morning (08h00–10h00) versus afternoon (16h00–18h00) RT [295]. The subgroup of patient receiving high dose RT developed less high-grade oral mucositis and less weight loss when RT was administered in the morning (more cells in G1 phase, more radioresistant) versus in the late afternoon (more cells in G2/M, more radiosensitive). When gender differences were explored, the incidence of high-grade mucositis for morning versus afternoon RT for the 159 men (49.4% versus 64.1%, $p=0.078$) and 46 women (65.2% versus 56.5%, $p=0.76$) showed a trend in different directions with less mucositis for females receiving RT in the afternoon as opposed to the morning for males. The gender difference in RT toxicity suggested by the above study were confirmed in a recent study that contrasted morning versus afternoon RT in female patients with cervix cancer [296]. In this study, diarrhea due to RT to the lower gastrointestinal tract was less in females treated in the afternoon consistent with the oral mucositis data for females. This came as a surprise since the data for human oral mucosa cell cycle rhythms mentioned above are consistent in the timing of cell cycle events with studies documenting a circadian rhythm in mitotic activity and DNA synthesis in mouse tongue, esophagus, stomach, duodenum, jejunum, and rectum [297–299] and human rectal mucosa [183,184]. Thus it would have been predicted that morning RT would be associated with less toxicity in the small bowel and rectum.

4.2. Clock genes abnormalities in cancer tissue

In addition to host circadian variability, the molecular clock of the tumor is potentially even more variable between individuals, because of the accumulation of somatic mutations in cancer cells and their intrinsic genomic instability [300–307]. Core clock genes have been reported to display somatic mutations in one whole cancer genome study involving breast and colorectal cancers [308], but not in other studies involving kidney, brain, lung and pancreatic cancers [302–304,306,309–317] (Catalogue Of Somatic Mutations In Cancer [318–321], www.sanger.ac.uk/genetics/CGP/cosmic/, accessed on January 20th, 2010). More focused studies have described promoter hypermethylation as a more common epigenetic event causing core clock gene downregulation in human cancers [39,43,45,52,57,58,115,322]. The expression of clock genes has been studied in several cancer types and consistently found to differ from that in healthy nearby tissues of the same organ, collected at one time of the day (reviewed in [1,36,141]). *Per1* and/or *Per2* mRNAs are down-regulated in several cancers, including breast, lung, colorectal, pancreatic, endometrial and myeloid neoplasms [1,36,141]. This was confirmed using the Oncomine platform (www.oncomine.org/; accessed on January, 18th, 2010) [323–325], that allowed us to compare the expression level of core clock genes between pairs of primary tumor and the corresponding normal tissue from the same patient in large databases. Forty-nine datasets had cancer versus normal tissue data and dataset size of ≥ 75 samples [326–374]. We found no difference in the expression levels of *Bmal1*, *Clock*, *Npas2*, *Cry1*, *Cry2*, *Dec2*, *Timeless* and *Rev-erb- α* . However, *Per1* ($p=0.011$), *Per2* ($p=0.042$), *Per3* ($p=0.0008$) and *Dec1* ($p=0.024$) were significantly down-regulated in cancer tissue compared with normal tissue. Both *Per1* and *Per2* have been shown to have tumor suppression activity in experimental models [42,375]. Down regulation of PER2 protein in the primary

colorectal lesion, of patients with metastatic colorectal cancer receiving oxaliplatin, 5-FU and folinic acid, was found to be an independent prognostic factor for poorer survival [59]. In this study the 67 patients whose primary tumor had more than 60% of PER2-labelled cells, had a 42% decrease in the risk of early death, and a 2.8 months longer median survival as compared to 66 patients with less than 10% of PER2-stained cells [59]. Furthermore experimental data in rodents support the role of *Per2* in colorectal cancer tumorigenesis [376,377], and *Per2* is the core clock gene most often mutated in human colorectal cancers [308].

A genotype single nucleotide polymorphism (SNP) in NPAS2 was shown to be associated with a 49% reduction in risk of breast cancer [109], but had no prognostic value in 348 patients with early breast cancer, receiving adjuvant postoperative treatment [378]. This SNP was not associated with a significant difference in NPAS2 expression [378]. On the contrary, in 287 patients from the same cohort, the expression level of NPAS2 mRNA, assessed in frozen specimens of the primary breast cancer, was significantly associated with progression-free and overall survival [378]. Thus, the third of patients with highest NPAS2 expression showed a 67% decrease in the risk of earlier progression and a 68% decrease in the risk of earlier death, independently of other known prognostic factors, as compared to the patients with the lowest tercile distribution of NPAS2 mRNA expression [378].

On the contrary, the higher the expression of *CRY1* mRNA in peripheral blood mononuclear cells of 116 untreated patients with chronic lymphocytic leukemia, the poorer the treatment-free survival. Low expression of *CRY1* in leukemic cells was independently associated with less aggressive clinical course of the disease [379]. The prediction of clinical aggressiveness was improved when *CRY1* expression was adjusted to *PER2* expression: a low (<0.16) *PER2*:*CRY1* ratio was independently associated with a 223% increase in the likelihood of longer time to starting treatment, as compared with high *PER2*:*CRY1* ratio [379].

The assessments of the functioning status of the molecular circadian clock of the tumor and of the circadian timing system of the host have already proven relevant for the identification of subgroups of patients with different therapeutic outcomes. Future studies might be able to use such data to guide personalized anticancer chronotherapy, by pinpointing patients most likely to benefit from a given chronotherapy schedule and/or a given chronobiotic or behavioral intervention.

4.3. Can we change the host rhythm to optimize therapy?

Rather than adapting a drug administration time to the inter-individual differences in circadian rhythms one could theoretically attempt to target the circadian timing system to reset it to optimize the therapeutic index of a given drug administered at a fixed time. In other words, the host circadian timing system could be modulated by pharmacologic means in order to strengthen its signal, reset its timing, shield it from treatment-induced disruption and even force a desynchronization between tissues. While there are no clinical data at this point, potentially useful drugs are already available or in development. Chronobiotic drugs could target the central pacemaker, the suprachiasmatic nuclei, its output signals to peripheral clocks, or the molecular clock machinery itself. The SCN neurons receive synaptic and humoral signals from other brain areas which involve several neuromediators, including serotonin (5-HT), glutamate (glu), neuropeptide Y (NPY), neurokinin-1 (NK-1), neuropeptide Y (NPY), pituitary adenylate cyclase-activating polypeptide (PACAP) and melatonin [380]. Selective antagonists and/or agonists exist for many of the receptors of these mediators that are used in other clinical indications such as the antiemetic NK-1 receptor antagonists aprepitant and casopitant [381–385]. A novel melatonin receptor 1 and 2 agonist, tasimelteon has shown good results for the treatment of transient insomnia

associated with jet-lag, by inducing a shift in endogenous melatonin rhythm [381]. The SCN rhythmically releases diffusible factors which provide internal synchronisation of peripheral oscillators. At least three substances have been identified so far; transforming growth factor- α (TGF- α), prokineticin-2 (PK-2) and cardiotrophin-like cytokine (CLC) [386–388]. The first one, TGF- α , is particularly interesting since its receptor is the epidermal growth factor receptor 1 (EGFR1, HER-1), that is a therapeutic target for several compounds that have been used for cancer therapy [389]. The potential impact of tyrosine kinase inhibitors that block EGFR on the host circadian timing system has been reviewed [390,391], and the effect of one of these molecules, gefitinib, on the circadian rest-activity rhythm of patients with advanced lung cancer has already been reported [392].

The molecular clocks in peripheral organs are sensitive to circulating hormones, with a certain degree of tissue specificity. For example, exogenous glucocorticoid analog administration has been shown to transiently change the phase of circadian gene expression in liver, kidney and heart, but not in neurons of the master clock [393,394]. Thus, glucocorticoid signalling may be used to force peripheral desynchronisation from the SCN.

High-throughput compound screening approaches have been recently developed in order to identify molecules capable of modifying the molecular clock, especially by inhibiting post-translational modifications of core clock proteins, and several chemical probes are under development for different therapeutic indications, including circadian disorders and cancer [14,395–397]. Seliciclib, a cyclin-dependent kinase inhibitor, is currently being investigated in phase-II and III trials [398]. This molecule has inhibiting activity against casein kinase I ϵ . Murine studies have shown that the therapeutic index for Seliciclib is optimized with administration at a time of day that is associated with nearly-physiologic tumor molecular clock rhythms [395]. Alongside casein kinase I ϵ , at least 6 other kinases have been shown to phosphorylate core molecular clock proteins in mammals, thus modifying their abundance, activity and subcellular localization. Glycogen synthase kinase 3- β , casein kinase 1- δ and epsilon, casein kinase 2- α , receptor for activated C kinase 1, protein kinase C- α and dual-specificity tyrosine-phosphorylated and regulated kinase 1A are all involved in post-translational regulation of the molecular clock, with specific phenotypic effects resulting from their knock-out. The pharmacologic inhibition of these kinases may have clinical implications [399–404]. Recent genome-wide siRNA screening has identified a series of genes involved in various pathways whose inhibition alters the function of the human molecular clock [405]. It is therefore possible that a pharmacologic modification of some molecular pathways may indirectly impact on the circadian clock. Some components of insulin and hedgehog signalling pathways, whose knock-down modulates the period or amplitude of the molecular circadian clock, may be particularly interesting [405]. Pharmacologic inhibitors of these pathways, used for their anticancer properties, are available or in advanced development [406–418].

Several pathways that are important in oncology [419–421], including cell cycle and the folate metabolism, involve a large number of genes that alter human oscillatory function [405]. Both of these pathways are targeted by common anticancer drugs [398,422–426], such as 5-FU, that has been shown to disrupt the molecular clock [427]. Furthermore, DNA damage, elicited by alkylating agents and platinum compounds, has been shown to have phase-advance properties [428].

4.4. Towards personalized chronochemotherapy

Personalized chronochemotherapy, with an optimal schedule adapted to each individual patient may become possible with coordinated and dedicated development of in vitro, in vivo and in silico models before clinical testing [1]. Current translational approaches under study in an effort to individualize cancer therapy

[429–462], should be broadened to include the acquisition of data from dynamic monitoring of relevant circadian biomarkers in individual patients [1]. The knowledge, derived from theoretical models, experimental and translational data, and novel programmable-in-time drug-delivery technologies, may enable a chronopharmacological approach that will improve the therapeutic index of cancer therapy with optimal clinical benefit for each individual cancer patient [1].

5. Conclusions

In this review we have discussed several studies, in different clinical settings, using various anticancer drug classes, which have been performed with the aim of improving the therapeutic index by accounting for the circadian stage of drug administration. Several complex methodological issues must be taken into account to make a valid comparison between conventional cancer drug scheduling and chronotherapy. Better measures of the individual differences in circadian biology of host and cancer are required to further optimize the potential benefit of chronotherapy for each individual patient. A dedicated drug developmental process is required to discover and validate optimal chronotherapy schedules in oncology. This must start at the preclinical level where drug-timing questions can be posed. If the preclinical data do suggest that therapy timing may be important, subsequent phase-I, phase-II and Phase-III development of new drugs should incorporate question on therapy timing. In some cases this could make the difference between the success or failure of new drugs [1].

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V.1.3. Article # 3, the Lancet Oncology, 2010

Régulation des rythmes circadiens et de l'axe hypothalamo-hypophyso-surrénalien : une interaction négligée dans le cancer.

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Les rythmes circadiens constituent une composante importante des interactions entre le système nerveux central et la tumeur. Les stratégies de prévention et de traitement des cancers pourraient largement bénéficier de la compréhension des effets favorables liés à la persistance des rythmes circadiens et des effets nocifs provoqués par une disruption circadienne. Une telle compréhension pourrait aussi révéler de nouvelles cibles thérapeutiques en cancérologie, spécifiques du système circadien.

These cyanine dyes have superior optical properties for tumour imaging owing to a rigid cyclohexenyl ring in the heptamethine chain with a central chlorine atom that maintains photostability, increases quantum yield, and decreases photobleaching. The dyes have superb biocompatible, pharmacokinetic, and retention properties. They can persist in tumours over 2 weeks for repeated imaging, but free dyes in circulation can be excreted rapidly from the interstitial fluid, resulting in no apparent acute toxic effect and improved signal contrast of tumours. The cyanine dyes can reach a contrast index value up to 20, whereas previously, a contrast index in a tumour of more than 2.5 times compared with that in its surrounding tissue was regarded as substantial accumulation.⁵ Additionally, the fluorescence of these dyes was stable after formalin fixation, which raised the possibility of developing new and sensitive means of detecting tumours in harvested surgical specimens.⁶

Most human tumours have been identified with higher mitochondrial membrane potentials than normal cells, making these dyes attractive imaging agents for cancer detection.⁷ Therefore, cyanine dyes can be used non-invasively to detect tumour and tumour metastases

in vivo, cancer cells in pathological specimens, and circulating cancer cells in blood to improve cancer detection, prognosis, and treatment.

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CS initially made the concept and identified these heptamethine cyanine dyes with native tumour targeting and near-infrared imaging properties. CS is also an inventor on the pending patents for these cyanine dyes as tumour imaging agents. CZ, YS, and TC declared no conflicts of interest.

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Regulation of circadian rhythms and hypothalamic-pituitary-adrenal axis: an overlooked interaction in cancer

An interesting Personal View in *The Lancet Oncology* by Katrina Ondicova and Boris Mravec¹ emphasised bidirectional interactions between peripheral tumour cells and the CNS and autonomic nervous systems. Here, we provide a complementary viewpoint to highlight other important cancer-relevant CNS functions such as the regulation of circadian rhythms and the hypothalamic-pituitary-adrenal axis.

Physiological circadian rhythms are coordinated by a hierarchical network, whose master pacemaker, the suprachiasmatic nucleus, is located in the anterior hypothalamus. This brain region, mentioned by Ondicova and Mravec,¹ is involved in homeostatic, behavioural, and neuroendocrine functions, with potential tumour-modulatory effects.²

In addition to the central pacemaker in the brain, every peripheral cell is equipped with a molecular clock, which

regulates daily oscillations in gene expression.³ The processes controlled by the molecular clock include cell-cycle gating, DNA repair, apoptosis, angiogenesis, and immune function, which are all noted in the Personal View as important mechanisms of cancer progression.^{2,3}

Research in experimental models and patients with cancer has shown that dysregulation of circadian physiological rhythms acts as an important inducer and promoter of cancer.^{2,3} Circadian rhythm dysregulation also exacerbates symptom burden and reduces health-related quality of life.²

Disrupted salivary cortisol, or locomotor circadian rhythms are associated with shorter survival in patients with breast or colorectal cancers, independent of other known prognostic factors.^{4,5} Studies have also shown that circadian rhythm dysregulation is a marker and potential mediator of cancer-relevant immunosuppressive or

immunodysregulatory effects of chronic stress.⁶ As a result of evidence from epidemiological and laboratory studies, the International Agency for Research on Cancer has determined that circadian rhythm disruption is probably carcinogenic to humans (group 2A).⁷

Ondicova and Mravec also discuss input signals from tumours that affect the CNS through the paraventricular hypothalamic nucleus.⁴ The paraventricular hypothalamic nucleus is anatomically and physiologically linked to the suprachiasmatic nucleus, and both nuclei are sensitive to blood-borne signals from peripheral organs.⁸ Tumour-associated pro-inflammatory cytokines are important signalling molecules involved in major cancer-related symptoms such as depression and the disruption of sleep and circadian rhythms.⁹ Sleep disruption in women with metastatic breast cancer is associated with reduced parasympathetic tone as measured by respiratory sinus arrhythmia,¹⁰ thus providing a connection between Ondicova and Mravec's observation of oncological effects of autonomic nervous system activity and circadian rhythm disruption.

In summary, we believe that the circadian rhythms of brain-body interactions are a major aspect of CNS-tumour crosstalk. Understanding of the beneficial health-promoting role of regulated circadian rhythms and the harmful effects of circadian dysregulation could improve cancer-prevention strategies and the pharmacological and behavioural treatment of cancer, and provide novel biological and behavioural targets for intervention.

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PFI is an employee of APHP, Paris, France, and PFI and FL received grant support from the European Commission. OP is an employee of the University of Rochester and received consultancy fees and grant support from NCI. OP also received fees for honoraria, travel, and accommodation from the Department of Defence, and payment for lectures and honoraria from Laval University, Canada. FSD received lecture honoraria, and grant support from NIH and NCI. FSD also received consultancy fees for other unrelated NIH projects. DS received grant support from NIH, National Centre for Complementary and Alternative Medicine, and an RO1 research grant on sleep and cancer from the National Institute of Cancer.

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V.2. Valeur pronostique de la qualité de vie et du rythme circadien pour la survie des patients atteints de cancer colorectal métastatique

V.2.1. Article # 4, Journal of Clinical Oncology, 2008

Validation du fonctionnement social estimé par le patient comme facteur pronostique indépendant de survie des patients atteints de cancer colorectal métastatique: résultats d'une étude internationale du Groupe de Chronothérapie de l'European Organisation for Research and Treatment of Cancer

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Une étude récente a identifié un modèle pronostique de la survie des patients atteints de cancer colorectal métastatique. Celui-ci comprenait la numération leucocytaire, les phosphatases alcalines, le nombre de sites métastatiques, et le fonctionnement social estimé par le patient. L'objectif de la présente recherche est de valider ce modèle sur les données d'une cohorte indépendante. Cette étude de validation est basée sur un essai prospectif randomisé et contrôlé chez des patients atteints de cancer colorectal métastatique menée par le Groupe de Chronothérapie de l'European Organisation for Research and Treatment of Cancer (EORTC). Globalement, 564 patients ont été inclus dans 10 pays. Pour cette validation indépendante, les patients avec les données de qualité de vie liée à la santé (QdVLS) avant traitement sont pris en compte. La QdVLS a été évaluée en utilisant le questionnaire de qualité de vie de l'EORTC QLQ-C30. Le modèle de régression de Cox a été utilisé pour les analyses de survie uni- et multi-variées. Le modèle précédent a été reproduit, avec un ajustement supplémentaire, la stratification selon le sexe. Les paramètres du modèle ont été confirmés comme facteurs pronostiques indépendants pour la survie: la numération leucocytaire, avec un

hazard ratio (HR) de 1,31 (IC 95%, de 1,021 à 1,698; P= 0,034); les phosphatases alcalines, avec un HR de 1,53 (IC 95%, de 1,188 à 1,979, P = 0,001); le nombre de sites métastatiques, avec un HR de 1,90 (IC 95%, de 1,531 à 2,364, p <0,0001), et le fonctionnement social estimé par le patient, avec un HR de 0,94 (IC 95%, 0,905 à 0,976, p = 0,001). Ceci se traduit par une augmentation de 6% de la probabilité de décès précoce pour chaque diminution de 10 points dans l'échelle de fonctionnement social de l'EORTC QLQ-C30. Cette étude confirme la valeur pronostique indépendante de la dimension sociale de la Qualité de Vie estimée par les patients atteints de cancer colorectal avancé.

Validation of Patient's Self-Reported Social Functioning As an Independent Prognostic Factor for Survival in Metastatic Colorectal Cancer Patients: Results of an International Study by the Chronotherapy Group of the European Organisation for Research and Treatment of Cancer

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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ABSTRACT

Purpose

A recent study identified a prognostic model for survival in metastatic colorectal cancer patients which included WBC count, alkaline phosphatase (AP), number of metastatic sites, and patients' self-reported social functioning. The aim of this research is to validate this model on data from an independent sample.

Patients and Methods

This validation study is based on a prospective randomized controlled trial in patients with metastatic colorectal cancer conducted by the European Organisation for Research and Treatment of Cancer (EORTC) Chronotherapy Group. Overall, 564 patients in 10 countries were enrolled. For the purpose of this independent validation, patients with health-related quality of life (HRQOL) baseline data were analyzed. HRQOL was assessed using the EORTC Quality of Life Questionnaire C30 (QLQ-C30). The Cox proportional hazards regression model was used for both univariate and multivariate analyses of survival.

Results

The previous model with an additional adjustment, by stratification for sex, was replicated and its parameters were confirmed to independently predict survival: WBC count with a hazard ratio (HR) of 1.31 (95% CI, 1.021 to 1.698; $P = .034$); AP with an HR of 1.53 (95% CI, 1.188 to 1.979; $P = .001$); number of sites involved with an HR of 1.90 (95% CI, 1.531 to 2.364; $P < .0001$); and patients' self-reported social functioning with an HR of 0.94 (95% CI, 0.905 to 0.976; $P = .001$). The latter translates into a 6% increase in the likelihood of an earlier death for every 10-point decrease in the social functioning scale of the EORTC QLQ-C30.

Conclusion

This study provides confirmatory evidence of the independent prognostic value of patients' self-reported social functioning in patients with advanced colorectal cancer.

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INTRODUCTION

A growing body of literature is showing that patients' perception of their own health status, as measured by multidimensional measures of health-related quality of life (HRQOL), can provide independent prognostic information beyond that of traditional biomedical data. The identification of patient-reported HRQOL parameters with independent prognostic value for survival outcomes, for example, could have important implications for routine clinical practice and clinical research. This information could help facilitate

the routine treatment decision-making process, perhaps enabling physicians provide patients with more timely treatment interventions. It could also help to better stratify patients included into randomized controlled trials.

The potential value of HRQOL as a key prognostic factor has recently emerged in more than 50 studies.¹ This evidence has been shown in several advanced cancer populations including bladder,² breast,^{3,4} colorectal,^{5,6} esophagogastric,^{7,8} head and neck,^{9,10} hepatocellular,¹¹ melanoma,¹² myeloma,^{13,14} and lung cancer.¹⁵⁻¹⁷ However, findings regarding which specific HRQOL parameter is the

best predictor in a given cancer population need to be confirmed in independent samples as different results have been observed and no independent validation studies have been published. It is crucial to validate previously constructed prognostic models on independent samples of patients.^{18,19}

A recent large study (European Organisation for Research and Treatment of Cancer [EORTC] 40952)⁶ showed the independent prognostic value of pretreatment patients' self-reported social functioning in a population of chemotherapy-naïve patients with advanced colorectal cancer treated with fluorouracil (FU)-based chemotherapy. The final multivariate model of this study also identified WBC count, alkaline phosphatase, and number of metastatic sites to independently predict overall survival (OS).⁶ This final model was also internally validated using a bootstrap resampling procedure to investigate model selection stability²⁰ confirming this one as being the most adequate model.⁶

The objective of this study is to validate the above reported prognostic model (EORTC 40952)⁶ on an independent data set of patients with similar baseline characteristics and to confirm the independent prognostic value of patients' self-reported social functioning. To our knowledge, this is the first validation study investigating the independent prognostic value of a patient's self-reported HRQOL parameter using exactly the same methodology on an independent sample of patients with cancer.

PATIENTS AND METHODS

Study Design

This work is based on data from a prospective multicenter randomized controlled trial in previously untreated patients with metastatic or recurrent colorectal cancer, conducted by the EORTC Chronotherapy Group (EORTC study 05963).²¹ Overall, 564 patients from 36 centers in 10 countries were enrolled. Two hundred eighty-two patients were randomly assigned to receive a chronomodulated infusion of FU, leucovorin, and oxaliplatin for 4 days (ChronoFLO4) and 282 patients received a conventional 2-day delivery of the same drugs (FOLFOX2). The primary end point of the trial was 2-year survival rate; HRQOL was a secondary end point. Median survival did not differ significantly between both treatment arms. However, a significant and independent sex per treatment schedule interaction was demonstrated. Women treated with ChronoFLO4 had a median survival that was significantly poorer than those receiving FOLFOX2 ($P = .03$), while men treated with ChronoFLO4 had significantly better survival than those receiving FOLFOX2 ($P = .02$). Full details have been previously reported.²¹ Given the above results, stratification for treatment arm and sex was added for all survival analysis in this validation study. We used the HRQOL data collected at baseline in this study as a validation data set for the multivariate prognostic model that had been previously developed for the EORTC 40952 study.⁶

Patients

Main eligibility criteria included: histologic proof of diagnosis of colorectal adenocarcinoma; age ranging between 18 to 76 years; adequate hematologic, renal, and hepatic functions; measurable metastatic lesions; no brain metastases; and no previous chemotherapy for metastatic disease (with the exception of previous adjuvant treatment if it was completed at least 6 months before inclusion). Patients were required to have a WHO performance status of 2 or less. The study, approved by the EORTC protocol review committee and the ethics committee of each participating center, was conducted in compliance with the Helsinki declaration. All patients provided written informed consent.

HRQOL Assessment

Patients' health status was measured using a multidimensional questionnaire of HRQOL—EORTC Quality of Life Questionnaire C30 (QLQ-C30),

version 2.0.²² This is an internationally validated HRQOL questionnaire suitable for use with a generic cancer population. It is available in numerous languages and has proven robust psychometric properties.²² Assessments were performed at baseline considering a time window of 15 days before or after randomization, but in any case before treatment start. Wherever possible, the questionnaires were administered at the clinic, in a room where the patient would not be disturbed. EORTC guidelines for administering questionnaires were provided, ensuring a standard approach to the collection of HRQOL data. The EORTC QLQ-C30 scores were calculated using the recommended EORTC procedures.²³ These involved transformation of raw scores into a linear scale ranging from 0 to 100. In the case of missing items within a scale, the scale score was calculated using only those for which values were available, provided at least half of the items in the scale were completed.

Ten scales of the EORTC QLQ-C30, as previously identified in the original study (EORTC 40952),⁶ were selected a priori for this analysis: physical, emotional, and social functioning; fatigue; nausea/vomiting; pain; appetite loss; constipation; diarrhea; and the global health status/QOL scale. These baseline HRQOL parameters were also compared with the original study (EORTC 40952)⁶ to investigate if there were any differences in the two populations in terms of HRQOL.

Patient Demographics and Biomedical Data

All the previously identified clinical parameters in the original study (EORTC 40952)⁶ were included in the analysis with the same cutoff criteria wherever applicable: performance status (continuous), WBC count (cutoff value: $10 \times 10^9/L$), alkaline phosphatase (cutoff value: 300U/L), number of metastatic sites involved (continuous), presence of liver metastases (yes v no), previous adjuvant chemotherapy (yes v no), and primary site of disease (colon v other). These biomedical characteristics were compared between the two study populations in order to document any potentially relevant difference between the original study (EORTC 40952)⁶ and the current validation one (EORTC 05963).

Statistical Analysis

OS was measured from the date of randomization to the date of death (due to any cause). Patients still alive at the time of analysis were censored at the last date known to be alive. Survival curves and probabilities were estimated using the Kaplan-Meier technique.²⁴ Differences between survival curves were assessed using the log-rank test.²⁵ The Cox proportional hazards regression model was used for both univariate and multivariate analyses of survival.²⁶ For the analysis of prognostic factors for survival, the proportionality assumption was checked for each of the variables under study by testing the dependency of their hazard ratio (HR) over time.²⁷ In the Cox models, all of the preselected HRQOL scales were included as continuous factors, rescaled on a 0 to 10 range, using data from baseline assessments. In order to adjust for potential predictive factors without including these in the model, treatment and sex were taken as stratification factors. A sensitivity analysis, using only treatment as stratification in the multivariate Cox model, was also fitted to directly compare outcomes with that of the original study (EORTC 40952).⁶ The importance of a prognostic factor was assessed via Wald-type test statistics, the HR, and its 95% CI for survival. A level of 5% of significance was used for both biomedical and HRQOL variables. In order to investigate the strength of the association, a Pearson's correlation matrix was also performed to investigate the association among all baseline variables used in the analysis. Discrimination C-indexes¹⁸ were computed to quantify the predictive accuracy of a model. All data analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Main Biomedical Characteristics of Study Populations

Of the 564 patients enrolled in the EORTC 05963 trial, 443 provided valid HRQOL baseline questionnaires (78.5%), which were used for the purpose of this study. There were no differences in the main clinical characteristics between patients with and without HRQOL baseline data, as well as no difference in terms of OS, the

median being 19.5 and 18.7 months, respectively ($P = .193$). Of the 443 patients, 220 received FOLFOX2 (49.7%) and 223 received ChronoFLO4 (50.3%). With 354 observed deaths and a reported overall median survival time of 19.5 months, the data were sufficiently mature to conduct an adequately powered analysis. The proportional hazards assumptions were found to be valid for the selected prognostic variables.

Most of the clinical characteristics of patients enrolled in this validation study (EORTC 05963) were similar to those in the original study (EORTC 40952).⁶ However, in this validation study, fewer patients were accrued with rectal primary tumors (24% v 50%) and more patients were accrued with a single metastatic site (50% v 27%). Median survival of patients enrolled in this confirmatory study was higher, being 19.5 months (95% CI, 18.3 to 20.8 months) compared with the median survival of patients in the original study (EORTC 40952)⁶ where the median survival was 14.2 months (95% CI, 12.1 to 16.2 months). Details are reported in Table 1.

Variable	Original Study (n = 299)		Validation Study (n = 443)	
	No.	%	No.	%
Median age, years	61.7		62.0	
Range	29.4-76.1		22.0-75.0	
Sex				
Male	180	60.2	265	59.8
Female	118	39.5	178	40.2
Missing	1	0.3	0	
WHO performance status				
0	157	52.5	209	47.2
1	121	40.5	188	42.4
2	19	6.4	46	10.4
Missing	2	0.7	0	
Adjuvant chemotherapy				
Yes	40	13.4	79	17.8
No	258	86.3	364	82.2
Missing	1	0.3	0	
Liver metastases				
Yes	246	82.3	379	85.6
No	53	17.7	63	14.2
Missing	0		1	0.2
Site of primary tumor				
Colon	148	49.5	333	75.2
Rectum	150	50.2	106	23.9
Other	1	0.3	4	0.9
No. of sites involved				
1	80	26.8	221	49.9
> 1	219	73.2	222	50.1
WBC				
≤ 10 × 10 ⁹ /L	231	77.3	343	77.4
> 10 × 10 ⁹ /L	68	22.7	100	22.6
Alkaline phosphatase, U/L				
≤ 300	210	70.2	342	77.2
> 300	89	29.8	101	22.8
Survival, months				
Median	14.2		19.5	
95% CI	12.1 to 16.2		18.3 to 20.8	

Abbreviation: EORTC, European Organisation for the Research and Treatment of Cancer.

HRQOL Baseline Characteristics of Study Populations

HRQOL scores in this population were very similar to those of the original study (EORTC 40952),⁶ without any clinically meaningful difference (ie, > 10 points²⁸) in the selected scales of the EORTC QLQ-C30 between the two populations (Table 2). In both studies, patients reported near identical scores (± standard deviation) on the social functioning scale, these being 75.7 (± 29.1) and 75.4 (± 29.2) for this validation and the original study (EORTC 40952),⁶ respectively.

To investigate the magnitude of the association between social functioning and other outcomes in both studies, pairwise correlations were calculated and results are reported in Table 3.

Distribution of HRQOL scores was also investigated in the two treatment arms (ChronoFLO4 v FOLFOX2) and in both sexes of patients included in the validation data set and these were similar (data not shown).

Relationships Between Patient-Reported HRQOL Parameters and Survival (Survival Prognostic Model)

All the preselected HRQOL parameters, apart from constipation and diarrhea, were prognostic in the univariate analysis. Also, all the usual biomedical prognostic factors for survival including performance status, number of sites involved, WBC count, and alkaline phosphatase predicted survival in this study (Table 4).

The final multivariate model previously identified in the original study (EORTC 40952)⁶ was applied to this independent sample (EORTC 05963). All of the traditional parameters previously identified to be independent prognostic factors (ie, WBC count, alkaline phosphatase, and number of metastatic sites) were confirmed in this validation data set. Also, patients' self-reported social functioning was confirmed to be a key independent prognostic factor with an HR of 0.94 (95% CI, 0.905 to 0.976; $P = .001$) and a 10-point shift worse on this scale translated into 6% increase in the likelihood of an earlier death. Details on comparisons of the two prognostic models are reported in Table 5. A sensitivity analysis using only treatment as stratification factor in the final Cox model yielded similar results. The HRs of an earlier death corresponding to baseline social functioning were similar ($P < .01$) whether Cox analyses were performed with stratification for treatment (HR, 0.941), for treatment and sex (HR, 0.940), or without such stratifications (HR, 0.943). Harrell's C-indices¹⁸ were also calculated for the presented Cox model with and without social functioning as a factor, revealing an improvement from 0.629 to 0.648 in predictive power. The C-indices for the model without sex and/or treatment as stratification factors remained unchanged at 0.648 indicating there was no inflation due to the stratification.

Survival curves based on social functioning scale terciles are reported in Figure 1 for descriptive purposes. Patients scoring below the lower tercile functioning score (< 60 points) had a median survival of 13.5 months as compared with patients scoring in the middle (≥ 60 and < 100) or the upper tercile (= 100), who had a median survival of 20.9 and 20.8, respectively (overall test, $P < .001$). This translated into a 2-year survival rate of 22% versus 42% versus 43% for the lower, middle, and upper tercile of the social functioning score, respectively. Details are reported in Figure 1.

DISCUSSION

The independent prognostic value of patients' self-reported social functioning, estimated with the EORTC QLQ-C30, was validated on a

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Table 2. Comparison of Baseline HRQOL Scores of Patients Enrolled in the Original Study (EORTC 40952)⁶ and Those Enrolled in this Validation Study (EORTC 05963)

Study	Patient-Reported HRQOL Parameter									
	Global Health Status/QOL	Physical Functioning	Emotional Functioning	Social Functioning	Fatigue	Nausea/Vomiting	Pain	Appetite Loss	Constipation	Diarrhea
Validation (N = 443)										
No.	437	442	443	440	441	443	443	440	440	439
Mean	61.65	76.52	70.20	75.72	36.38	8.09	22.91	23.26	17.80	10.78
SD	22.04	26.63	23.28	29.13	27.01	18.23	27.34	31.53	28.26	20.28
Original (N = 299)										
No.	293	297	296	296	297	298	298	296	294	294
Mean	58.56	76.89	66.25	75.45	37.28	6.77	26.06	21.96	13.49	14.85
SD	22.10	24.23	25.49	29.17	29.16	15.29	30.75	30.84	26.47	27.03

Abbreviations: EORTC, European Organisation for the Research and Treatment of Cancer; HRQOL, health-related quality of life; QOL, quality of life; SD, standard deviation.

large and independent population of patients with advanced colorectal cancer. At baseline, this variable investigates how the disease burden has limited a patient's social interactions with the following two questions: "has your physical condition or medical treatment interfered with your family life?" and "has your physical condition or medical treatment interfered with your social activities?"

While previous prognostic factor studies have shown the independent prognostic value of patients' judgment of their own health status for survival in various advanced cancer diseases, to our knowledge, no validation study has as yet been published on an independent sample involving a similar cancer patient population and using the same HRQOL multidimensional questionnaire. This confirmatory study therefore provides robust supportive evidence that social func-

tioning represents an important prognostic variable in patients with advanced colorectal cancer.⁶

This study has a number of strengths including a large international sample of patients (N = 443) recruited in a multicontinental prospective randomized trial with sufficient follow-up and mature survival data allowing for an adequately powered analysis. There was no difference in terms of survival between patients with and without baseline HRQOL data, thus ensuring the population analyzed for this validation study was representative of the overall trial population (EORTC 05963). Data collection in this sample was of high quality with HRQOL measured with a psychometric robust questionnaire and main clinical data available for all patients.

In addition, the biomedical characteristics and HRQOL data were similar both in this study population and in that of the original

Table 3. Pearson Correlation Coefficients Between Patient's Self-Reported Social Functioning and Other Variables for the Original Study (EORTC 40952)⁶ and This Validation Study (EORTC 05963)

Variable	Validation Study		Original Study	
	Coefficient	No. of Patients	Coefficient	No. of Patients
Clinical parameter				
Performance status	-0.368	440	-0.256	294
No. of sites involved	-0.130	440	-0.006	296
WBC	-0.097	440	-0.043	296
Alkaline phosphatase	-0.088	440	-0.058	296
Liver metastases	-0.008	439	0.145	296
Adjuvant chemotherapy	0.109	440	0.021	295
Site of primary tumor	0.007	440	-0.080	295
HRQOL parameter				
Global health status/QOL	0.586	434	0.440	293
Physical functioning	0.473	439	0.432	294
Emotional functioning	0.418	440	0.529	296
Fatigue	-0.634	438	-0.495	295
Nausea/vomiting	-0.308	440	-0.187	296
Pain	-0.527	440	-0.484	296
Appetite loss	-0.516	437	-0.283	294
Constipation	-0.138	437	-0.226	294
Diarrhea	-0.264	436	-0.123	294

Abbreviations: EORTC, European Organisation for the Research and Treatment of Cancer; HRQOL, health-related quality of life; QOL, quality of life.

Table 4. Univariate Cox Regression Analyses of Survival Stratified by Treatment and Sex

Variable	Hazard Ratio	95% CI	P
Clinical parameter			
Performance status	1.590	1.356 to 1.865	< .0001
No. of sites involved	1.934	1.561 to 2.395	< .0001
WBC	1.579	1.240 to 2.011	.0002
Alkaline phosphatase	1.671	1.311 to 2.130	< .0001
Liver metastases	0.966	0.717 to 1.302	.820
Adjuvant chemotherapy	1.049	0.798 to 1.378	.733
Site of primary tumor	1.202	0.944 to 1.530	.134
HRQOL parameter*			
Global health status/QOL	0.914	0.872 to 0.959	.0002
Physical functioning	0.933	0.897 to 0.970	.0005
Emotional functioning	0.954	0.913 to 0.998	.038
Social functioning	0.922	0.888 to 0.956	< .0001
Fatigue	1.101	1.059 to 1.144	< .0001
Nausea/vomiting	1.126	1.065 to 1.191	< .0001
Pain	1.101	1.059 to 1.145	< .0001
Appetite loss	1.106	1.072 to 1.142	< .0001
Constipation	1.036	0.999 to 1.074	.056
Diarrhea	1.024	0.970 to 1.081	.390

Abbreviations: HRQOL, health-related quality of life; QOL, quality of life.

*Hazard ratios are reported for every 10-point shift difference on the scale (all scales range from 0 to 100).

Table 5. Final Cox Multivariate Regression Model of Survival for the Original Study (EORTC 40952)⁶ and this Validation Study (EORTC 05963)

Variable	Original Study*	Validation Study†
No.	296 of 299	440 of 443
WBC count		
Hazard ratio	1.961	1.317
95% CI	1.439 to 2.672	1.021 to 1.698
P	< .001	.034
Alkaline phosphatase		
Hazard ratio	1.509	1.533
95% CI	1.126 to 2.022	1.188 to 1.979
P	.005	.001
No. of sites involved		
Hazard ratio	1.108	1.903
95% CI	1.024 to 1.198	1.531 to 2.364
P	.010	< .0001
Social Functioning‡		
Hazard ratio	0.916	0.940
95% CI	0.876 to 0.958	0.905 to 0.976
P	< .001	.001

Abbreviation: EORTC, European Organisation for the Research and Treatment of Cancer.

*Stratified by treatment.

†Stratified by treatment and sex.

‡The hazard ratio is reported for every 10-point shift difference on the scale ranging from 0 to 100.

study (EORTC 40952),⁶ thus lending credit to our attempt to validate the previously identified multivariate model.

The proportion of patients with more than one metastatic site was inferior (50% v 73%) and oxaliplatin was added to FU and leucovorin in this study as compared with the original sample.⁶ These

differences most likely accounted for the OS differences between both trials. The median survival of patients was 14.2 months in the original study (EORTC 40952)⁶ and 19.5 months in this sample. The reduction in the patients' risk of death for any 10-point increase in social functioning scale (ie, a better score) was 9% in the original study (EORTC 40952)⁶ and 6% in this confirmatory study. This slight decrease in the HR could be influenced by the different median survival of patients included in this trial when compared with those in the previous one (19.5 v 14.2 months). This finding could be supported by previous evidence suggesting that HRQOL parameters are more likely to provide independent prognostic information in advanced cancer diseases, but not in patients with earlier stage disease.²⁹⁻³² Previous work has discussed possible mechanisms underlying the association between patients' self-perception of HRQOL parameters and survival outcomes.³⁰⁻³⁴

While the relationship between social functioning and survival of patients with colorectal cancer is not immediately clear, there is previous evidence suggesting that social functioning is a sensitive aspect of HRQOL in this cancer population. Anthony et al³⁵ investigated the prognostic value of a number of pretreatment HRQOL parameters for surgical complications in colorectal patients undergoing open surgical resection for colorectal cancer and found that an improved social functioning score (measured by the Medical Outcomes Study Short Form-36) was independently associated with a decreased likelihood of surgical complications in the multivariate analysis. Arndt et al³⁶ compared HRQOL in patients with colorectal cancer 1 year after diagnosis with that of a general population using the same measure of our study (the EORTC QLQ-C30) and showed that social functioning was the most impaired HRQOL domain out of all the other functional and global health status scales. With the aim of investigating whether HRQOL limitations continued to persist over a longer period, the

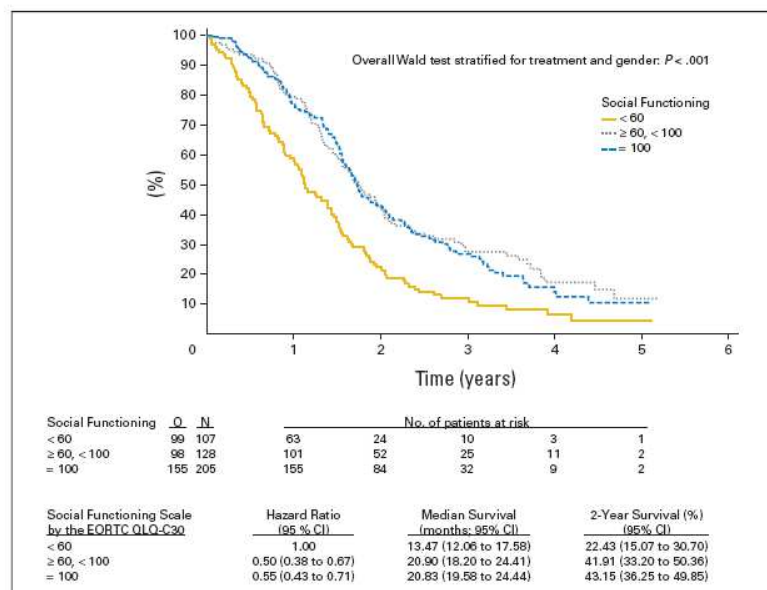


Fig 1. Overall survival curves in the three groups defined by Social Functioning scale of the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTC QLQ-C30) by tertiles. N, number of patients; O, number of observed events (ie, deaths).

same authors conducted a population-based study investigating this issue 3 years after diagnosis of colorectal cancer and confirmed that social functioning was still the most affected HRQOL domain out of the remaining functional and global health status scales measured with the EORTC QLQ-C30.³⁷ Overall, these results are in line with our findings—in our validation sample, social functioning was also the most impaired aspect out of all the other functional and global health status scales when compared with data of a general population. The mean social functioning score in our study was 15.3 points poorer than that found in a general noncancer population.³⁸

Our study has limitations. Given the design of our project, we were not able to investigate further the nature of the link between a patient's self-reported social functioning and survival. It is also possible that other different variables than social functioning would have emerged in the multivariate analysis had one been undertaken de novo rather than entering the variables from the final multivariate model identified in the original study (EORTC 40952).⁶

Although future research could investigate the underlying mechanism of the association between social functioning and length of survival, our data indicate that, in the very least, this specific HRQOL domain seems to capture the whole burden of the disease in a way which is not usually assessed by traditional medical indices commonly used in routine oncology practice. In our study, there were no major correlations between patients' self-reported social functioning and other traditional clinical data, however, the highest correlation was found with performance status. The evidence that a patient's own health status judgment provides a unique perspective, as well as independent prognostic information, could also greatly further support the need to move toward a more patient-centered approach as has also been recently highlighted to be crucial in the context of quality care.³⁹

In conclusion, for the first time the independent prognostic value of patients reported social functioning at baseline has been observed

and now confirmed in two separate, international, prospective large data sets of chemotherapy-naïve patients with advanced colorectal cancer. Therefore, present findings support the investigation of underlying biologic mechanisms, as well as the collection of patient-reported HRQOL data in routine clinical practice, because these could provide valuable prognostic information.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Le rythme circadien d'activité-repos: corrélat biologique de qualité de vie et facteur prédictif de survie des patients atteints de cancer colorectal métastatique

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Le rythme circadien d'activité et repos est un biomarqueur de la coordination centrale du système circadien. Dans une étude unicentrique réalisée dans notre Unité, la perturbation de ce rythme, enregistré pendant 48 h à 7 j, est quantifiée d'après des indices robustes d'analyse de reproductibilité sur 24 h (autocorrélation, r_{24}) et de dichotomie ($I < O$, rapport entre activités « au lit » et « hors du lit »). Chez 192 patients recevant une chimiothérapie chronomodulée pour cancer colorectal métastatique (CCRM) antérieurement traité, ces deux paramètres constituaient un facteur prédictif de réponse tumorale et un facteur pronostique de survie, indépendamment des facteurs pronostiques connus. Ces deux paramètres étaient aussi significativement corrélés à plusieurs dimensions de qualité de vie (QdV), identifiés d'après le questionnaire EORTC-QLQ-C30. Cette étude prospective internationale a pour objectif la validation de ces résultats, et de leur pertinence chez des patients atteints de CCRM et n'ayant jamais reçu de chimiothérapie. Après étude du rythme d'activité-repos, ces patients recevaient une première ligne de chimiothérapie par 5-fluorouracile, leucovorine et oxaliplatine en perfusion conventionnelle ou chronomodulée, adaptée au rythme circadien, dans le cadre d'un essai randomisé. Cent trente patients de 9 institutions ont porté un accéléromètre de poignet pendant 3 j avant chimiothérapie, et 96 d'entre-eux ont complété le questionnaire EORTC QLQ-C30. Cette étude confirme la pertinence de $I < O$: ce paramètre est corrélé à la qualité de vie globale, aux dimensions physique et sociale de QdV, à la fatigue et à l'anorexie ($r > |0.25|$, $p < 0.01$). De plus, $I < O$ constitue un facteur pronostique indépendant de la survie globale avec un Risque Relatif de 0.94 ($p < 0.0001$). La confirmation des relations entre les paramètres du

rythme d'activité-repos, la QdV et la survie est en faveur du rôle du système circadien dans le contrôle de la progression tumorale clinique. Ces résultats répliquent chez l'Homme, l'accélération de la progression tumorale par la disruption expérimentale du système circadien, obtenue par destruction du pacemaker hypothalamique, le décalage horaire chronique ou la mutation du gène de l'horloge *Per2*. Ainsi, le système circadien représente une cible thérapeutique potentielle : les interventions qui en restaurent la fonction, pourraient en effet améliorer la qualité de vie et la survie des patients cancéreux.

Research Article

Circadian Rhythm in Rest and Activity: A Biological Correlate of Quality of Life and a Predictor of Survival in Patients with Metastatic Colorectal Cancer

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Abstract

The rest-activity circadian rhythm (CircAct) reflects the function of the circadian timing system. In a prior single-institution study, the extent of CircAct perturbation independently predicted for survival and tumor response in 192 patients receiving chemotherapy for metastatic colorectal cancer. Moreover, the main CircAct parameters correlated with several health-related quality of life (HRQoL) scales. In this prospective study, we attempted to extend these results to an independent cohort of chemotherapy-naïve metastatic colorectal cancer patients participating in an international randomized phase III trial (European Organisation for Research and Treatment of Cancer 05963). Patients were randomized to receive chronomodulated or conventional infusion of 5-fluorouracil, leucovorin, and oxaliplatin as first-line treatment for metastatic colorectal cancer. Patients from nine institutions completed the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-C30 and wore a wrist accelerometer (actigraph) for 3 days before chemotherapy delivery. Two validated parameters (I-O and r24) were used to estimate CircAct. Of 130 patients with baseline CircAct assessments, 96 had baseline HRQoL data. I-O was confirmed to correlate with global quality of life, physical functioning, social functioning, fatigue, and appetite loss ($r > |0.25|$; $P < 0.01$). I-O further independently predicted for overall survival with a hazard ratio of 0.94 ($P < 0.0001$). The associations between CircAct parameters, HRQoL, and survival, which were shown in this international study involving previously untreated metastatic colorectal cancer patients, confirm prior single-institution findings in mostly pretreated metastatic colorectal cancer

patients. The circadian timing system constitutes a novel therapeutic target. Interventions that normalize circadian timing system dysfunction may affect quality of life and survival in cancer patients. [Cancer Res 2009;69(11):4700–7]

Introduction

Most activities of daily living, such as locomotor activity, eating behavior, sleep/wakefulness, psychophysical performance, and mood, are regulated along the 24-h period by the circadian timing system (1–3). This system is constituted of molecular circadian clocks in peripheral tissues, the coordination of which is ensured by the suprachiasmatic nuclei, a central pacemaker located in the anterior hypothalamus (1–3). The alternation of light and darkness, social routine, and feeding schedules calibrate the circadian timing system to precisely 24 h by resetting the central pacemaker and the peripheral clocks, respectively (4). The suprachiasmatic nuclei coordinate the peripheral clocks through polysynaptic neuroanatomic pathways as well as through blood-borne cytokines and hormones (5, 6). The circadian rhythm in locomotor activity (CircAct) is a well-established marker of the function of the central pacemaker (7, 8) and can be continuously and noninvasively assessed in an objective, reliable, and validated manner with wrist actigraphy (9, 10).

The affective and constitutional symptoms, which tend to cluster in cancer patients, may be partly due to circadian disruption (5). Disrupted circadian function, objectively estimated with wrist actigraphy, has been found to correlate with subjective parameters, such as performance status (PS; refs. 11–13) and self-reported symptoms, particularly fatigue, poor sleep, appetite loss, and depression (12–15). Jetlag and shift work are associated with the same symptoms (16–19).

Abnormal circadian rhythms have been associated with higher risk of cancer development and more rapid cancer progression in both rodents and humans. In tumor-bearing rodents, disruption of the circadian rest-activity rhythm resulted in faster tumor growth and shorter survival (20, 21). Shift work, with the associated disruption of the circadian rhythm, is a significant and independent risk factor for the development of breast, colorectal, endometrial, and prostate cancers (22–26).

Note: This study was presented in part at the 41st American Society of Clinical Oncology Annual Meeting (Orlando, FL; 2005; abstracts 3553 and 8029).

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Abnormal circadian rhythms have been shown in cancer patients (27–29) and associated with poorer outcome. In women with metastatic breast cancer, a disrupted cortisol rhythm was associated with poorer survival (30). In a prior single-institution study, the extent of CircAct perturbation independently predicted for survival and tumor response in 192 patients receiving chemotherapy for metastatic colorectal cancer (31). Moreover, CircAct correlated with several health-related quality of life (HRQoL) scales (31, 32). In the present study, we attempted to extend these previous results to an independent cohort of chemotherapy-naïve patients with metastatic colorectal cancer participating in an international randomized phase III trial.

Materials and Methods

Study outline. The focus of this study was to correlate the circadian rest-activity rhythms of patients with advanced colorectal cancer with quality of life and survival. This open prospective study was a companion to an international, multicenter, randomized two-arm phase III trial [European Organisation for Research and Treatment of Cancer (EORTC) 05963] that compared two biweekly schedules of the combination of oxaliplatin, 5-fluorouracil, and leucovorin in 564 chemotherapy-naïve patients with metastatic colorectal cancer (33). Patients were randomized to receive either a conventional 2-day regimen (FOLFOX2) or a 4-day chronomodulated schedule (chronofLO4). Inclusion criteria were PS of ≤ 2 (WHO scale), ages 18 to 76 years, adequate hematologic, renal, and hepatic functions, no overt brain metastases, and no prior chemotherapy or radiotherapy for metastatic disease. The primary trial registered 564 patients at 36 institutions in 10 countries. The 9 institutions (in Belgium, Canada, France, and Italy) participating in this companion study entered 191 patients on the main trial from August 1999. This group of patients was eligible for the current study that was approved by the local ethics review boards. Clinical outcomes (overall survival, progression-free survival, tumor response, and toxicity grading) were evaluated as described previously (33).

CircAct and HRQoL assessments. To assess individual circadian rest-activity rhythm, a Mini-Motionlogger actigraph (Ambulatory Monitoring) was used. The actigraph is similar to a watch and is worn on the nondominant wrist. It contains a piezoelectric linear accelerometer to detect wrist movements and a memory chip for data storage. The user-defined time interval for the recording and count of activity level was 1 min. The actigraph was worn for at least 72 h continuously before the beginning of the first or second course of chemotherapy (9, 10, 31). All actigraphy time series were analyzed using a specific program (Acton 4 version 1.10; Ambulatory Monitoring) by one investigator. Two robust and well-characterized parameters were used to estimate the circadian activity pattern: the dichotomy index (I-O), which integrates the circadian regulation of sleep and takes into account the relative difference in activity between the rest and wakeful spans, and the autocorrelation coefficient at 24 h (r_{24}), a measure of the regularity and reproducibility of the activity pattern over a 24-h period from one day to the next (31). In case of a prominent circadian rhythm, I-O reaches 100% and r_{24} reaches 1. Average activity (meanAct) was calculated as the average number of wrist movements per minute throughout the recording time. All parameter values were computed for the whole monitoring period (72 continuous hours).

The EORTC Quality of Life Questionnaire-C30 (version 2.0) was completed before the first or second course of chemotherapy (34). All scores were calculated using the standard recommended EORTC procedures.

Statistical analyses. Means, medians, SDs, and interquartile ranges were used as summary statistics for the raw distribution of CircAct parameters. The normality of the CircAct parameters distribution was assessed with the Shapiro-Wilk test. The Wilcoxon two-sample test was used to assess the association between CircAct parameters and binary factors or outcomes. The Jonckheere-Terpstra test was computed to assess the association between

CircAct parameters and ordinal factors. Logistic regression was used to test whether each quantitative CircAct parameters predicted for the occurrence of severe toxic events.

The correlations among CircAct parameters, between CircAct parameters and continuous variables, as well as the correlation between CircAct parameters and baseline HRQoL scores were examined using the Spearman's rank correlation coefficient. Partial correlation coefficients were computed between CircAct parameters and HRQoL scores using age, body mass index, and hemoglobin concentration separately as controlling variables. Correlations were considered as relevant if $r > |0.25|$ and two-sided $P \leq 0.01$. Moreover, CircAct parameters were dichotomized according to their median (above and below median), and the HRQoL indices were compared in these two subgroups with the Mann-Whitney U test.

The effect size of the difference in HRQoL scores between the two CircAct subgroups was calculated as the difference in subgroup means divided by the pooled SDs. The threshold for clinical relevance was set at effect size ≥ 0.4 .

Because multiple analyses were done, and because the sample size of the current study was not computed with these endpoints in mind, the threshold for statistically significant differences was set at $P < 0.01$.

Progression-free and overall survival functions were estimated by the Kaplan-Meier's method. The survival curves for each CircAct parameter were drawn in groups of patients defined according to the quartiles of the distribution of the considered parameter. The survival curves were compared using the log-rank test. The association between each CircAct parameter as quantitative variable and progression-free or overall survival was also assessed with a simple Cox's regression model. Each CircAct parameter found significantly prognostic with the simple regression analysis at the 5% significance level was included in a multiple Cox's regression model and adjusted for age, gender, body mass index, study center, primary tumor site, prior adjuvant chemotherapy, and treatment arm together with clinically relevant prognostic factors (31, 35). Stepwise regressions at the 5% significance level were done, and the bootstrap resampling technique was used as an internal validation tool.

Results

Study compliance. A total of 130 patients (23% of the whole trial population) had their CircAct evaluated: 103 patients before treatment onset, 24 patients after the first course, and 3 patients after the second course of treatment. Considering the 191 potential patients available for study, the true baseline compliance was 68%. Of the 130 patients with baseline actigraphy monitoring, 96 (74%) patients also completed the EORTC Quality of Life Questionnaire-C30.

Representativeness of the current study population. The main clinical characteristics of the 130 patients in this companion study were comparable with those of the 564 patients registered in the main trial (Table 1). The current study population had baseline HRQoL scores, overall survival, progression-free survival, response rate, and grade 3 to 4 toxicity rate that did not significantly differ from those of the remaining trial population ($P \geq 0.10$; data not shown).

Distribution of circadian rest-activity rhythm parameters. The 24-h patterns in rest-activity displayed wide interindividual differences. For meanAct and r_{24} , the assumption of a normal distribution was not rejected ($P = 0.20$ and 0.19 , respectively). Mean and median meanAct were 105 and 106 counts/min, respectively (SD, 27; interquartile range, 41). For r_{24} , mean and median values were 0.38 and 0.37, respectively (SD, 0.17; interquartile range, 0.24). Conversely, the distribution of I-O was nonnormal ($P < 0.0001$) and skewed. Thus, median I-O was 97.0, whereas mean I-O was 94.3 (SD, 8.6; interquartile range, 6.8). The CircAct parameters I-O and r_{24} were positively correlated with each other ($r = 0.74$; $P < 0.001$)

Table 1. Main clinical features of the whole EORTC 05963 trial population according to the availability or not of actigraphy assessment at baseline

Variable	CircAct assessment available		Total (33)
	Yes (n = 130)*	No (n = 434)	n = 564
	n (%)	n (%)	n (%)
Treatment arm			
FOLFOX2	67 (51.5)	215 (49.5)	282 (50)
ChronoFLO4	63 (48.5)	219 (50.5)	282 (50)
WHO PS			
0	70 (53.8)	203 (46.8)	273 (48.4)
1	45 (34.6)	186 (42.9)	231 (41.0)
2	15 (11.5)	45 (10.4)	60 (10.6)
Age (y)			
Median	60	63	62
Range	22-76	24-76	22-76
Gender			
Male	74 (56.9)	264 (60.8)	338 (59.9)
Female	56 (43.1)	170 (39.2)	226 (40.1)
Body mass index (kg/m ²)			
≤18.4 (underweight)	4 (3.1)	19 (4.4)	23 (4.1)
18.5-24.9 (normal)	57 (43.8)	220 (50.7)	277 (49.1)
25.0-29.9 (overweight)	51 (39.2)	134 (30.9)	185 (32.8)
≥30.0 (obese)	17 (13.1)	59 (13.6)	77 (13.7)
Missing	0	2 (0.5)	2 (0.4)
Median	25.2	24.5	24.6
Range	16.7-39.1	14.9-42.9	14.9-42.9
Site of primary tumor			
Colon	96 (73.8)	329 (75.8)	425 (75.4)
Rectum	34 (26.2)	105 (24.2)	139 (24.6)
No. metastatic sites			
0	0 (0.0)	4 (0.9)	4 (0.7)
1	58 (44.6)	224 (51.6)	191 (33.9)
2	52 (40.0)	139 (32.0)	86 (15.2)
≥3	20 (15.4)	66 (15.2)	1 (0.2)
Unknown	0 (0.0)	1 (0.2)	
Organs involved			
Liver	106 (81.5)	377 (86.9)	483 (85.6)
≤25%	62 (47.7)	204 (47.0)	266 (47.2)
>25%	44 (33.8)	173 (39.9)	217 (38.5)
Lung	47 (36.2)	162 (37.3)	209 (37.1)
Analgesics at entry	26 (20.0)	56 (12.9)	82 (14.5)
Previous adjuvant treatment	23 (17.7)	79 (18.2)	102 (18.1)
WBC (10 ⁹ cells/L)			
Median	8.0	8.1	8.1
Range	3.6-25.5	3.1-27.6	3.1-27.6
≥10	30 (23.1)	102 (23.5)	132 (23.4)
Baseline HRQoL available	96 (74)	347 (80)	443 (79)

*Current study population.

and with meanAct ($r = 0.47$; $P < 0.001$ and $r = 0.46$; $P < 0.001$, respectively).

CircAct correlation with patient characteristics. Patients with good PS had significantly more robust CircAct in comparison with patients with poor PS (Fig. 1) for 1<O ($P = 0.01$), r24 ($P = 0.0014$), and, to a lesser extent, meanAct ($P = 0.047$). Thus, median 1<O and r24, respectively, decreased from 98.2 and 0.44 in the patients with PS = 0 ($n = 70$) to 95.7 and 0.36 in those with

PS = 1 ($n = 45$) and 95.3 and 0.24 in patients with PS = 2 ($n = 15$). Patients receiving analgesic treatment at study entry ($n = 26$) displayed significantly more perturbed CircAct compared with patients without painkillers for 1<O ($P = 0.004$), r24 ($P = 0.001$), and, to a lesser extent, meanAct ($P = 0.048$). Indeed, median 1<O was 95.3 and median r24 was 0.26 in patients on analgesics, whereas corresponding median values were 97.7 and 0.41, respectively, in patients not requiring analgesics. CircAct parameters did not

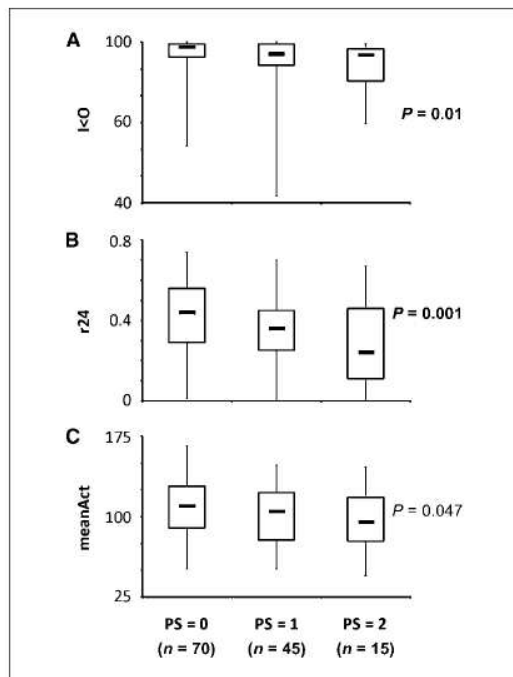


Figure 1. Distribution of the CircAct parameters I<O (A), r24 (B), and meanAct (C) according to the WHO PS. Box, median (middle line) and first and third quartiles; thin vertical columns, range.

correlate with age, gender, body mass index, percentage of liver involvement with tumor, number of metastatic sites, site of primary tumor, blood test results (blood counts, liver tests, and carcinoembryonic antigen), or previous adjuvant chemotherapy administration ($P > 0.10$).

CircAct correlations with HRQoL. All CircAct parameters (I<O, r24, and meanAct) correlated positively with global quality of life and role functioning but negatively with fatigue and appetite loss. In addition, I<O and r24 correlated positively with social functioning and negatively with pain and dyspnea. Furthermore, I<O correlated positively with physical functioning but negatively with insomnia. MeanAct correlated positively with physical functioning. Conversely, no CircAct parameter correlated with emotional or cognitive functioning, nausea/vomiting, constipation, or diarrhea. These correlations remained statistically significant after adjustment for age, with minimal modifications in coefficient values (range, -0.02 to +0.04) and P values (Table 2). Controlling for body mass index or hemoglobin level did not relevantly alter any of the previously listed correlations either (results not shown).

Figure 2 shows the difference in mean HRQoL scores between two subgroups of patients classified by CircAct parameters above median (black columns) versus below the median (gray columns). Based on the thresholds for clinical relevance (effect size ≥ 0.4 ; $P < 0.01$), higher than median I<O was associated with better global quality of life, higher role functioning, less fatigue, less appetite loss, and less pain. However, higher than median r24 was only

associated with less fatigue, whereas higher than median meanAct was associated with better global quality of life, higher role functioning, less fatigue, and better physical functioning.

CircAct correlations with toxicity, response, and survival. Baseline CircAct parameters predicted neither for best objective tumor response ($P \geq 0.14$) nor for the occurrence of at least one moderate or severe toxic event based on the National Cancer Institute of Canada Common Toxicity Criteria version 2.0 grading score ($P \geq 0.31$). No statistically significant association was found between progression-free survival and any CircAct parameter ($P > 0.10$).

For each CircAct parameter, the comparison of the survival functions of the patient groups defined by the quartiles of the parameter showed a significant difference for r24 ($P = 0.002$, log-rank test) and for I<O ($P = 0.008$, log-rank test) but not for meanAct ($P = 0.201$, log-rank test). The corresponding Kaplan-Meier survival estimate curves for r24 and I<O were plotted in Fig. 3A and B, respectively. No consistent trend in Kaplan-Meier survival estimates was observed as a function of r24 quartiles (Fig. 3A). Conversely, a trend was evident for I<O, with the poorest survival occurring in the lowest quartile group, intermediate and overlapping survival in both middle quartiles, and a slightly better survival in the highest quartile group (Fig. 3B). These observations were confirmed by plotting the log (-log) of the survival curves (data not shown).

A significant positive association was found between overall survival and I<O with a hazard ratio of 0.95 (95% confidence interval, 0.93-0.97; $P < 0.0001$) using a Cox's proportional hazards univariate regression model. A similar relationship was found for overall survival and r24, with a hazard ratio of 0.20 (95% confidence interval, 0.07-0.60; $P = 0.004$). No such association was found for survival and meanAct ($P = 0.15$). The relation between I<O and survival remained roughly similar following adjustment for the

Table 2. Partial correlation coefficients, adjusted for age, between HRQoL indices, and CircAct parameters at baseline, with significance levels

HRQoL scale (n = 96)	CircAct parameter		
	I<O	r24	meanAct
Global quality of life	0.39*	0.32 [†]	0.37*
Role functioning	0.36*	0.31 [†]	0.40*
Fatigue	-0.39*	-0.36*	-0.39*
Appetite loss	-0.32 [†]	-0.30 [†]	-0.32 [†]
Social functioning	0.35*	0.33*	±
Pain	-0.40*	-0.30 [†]	±
Dyspnea	-0.29 [†]	-0.27 [†]	±
Physical functioning	0.35*	±	0.35*
Insomnia	-0.33*	±	±
Emotional functioning	±	±	±
Cognitive functioning	±	±	±
Nausea/vomiting	±	±	±
Constipation	±	±	±
Diarrhea	±	±	±

* $P \leq 0.001$.

[†] $P \leq 0.01$.

± $r < [0.25]$ and/or $P > 0.01$.

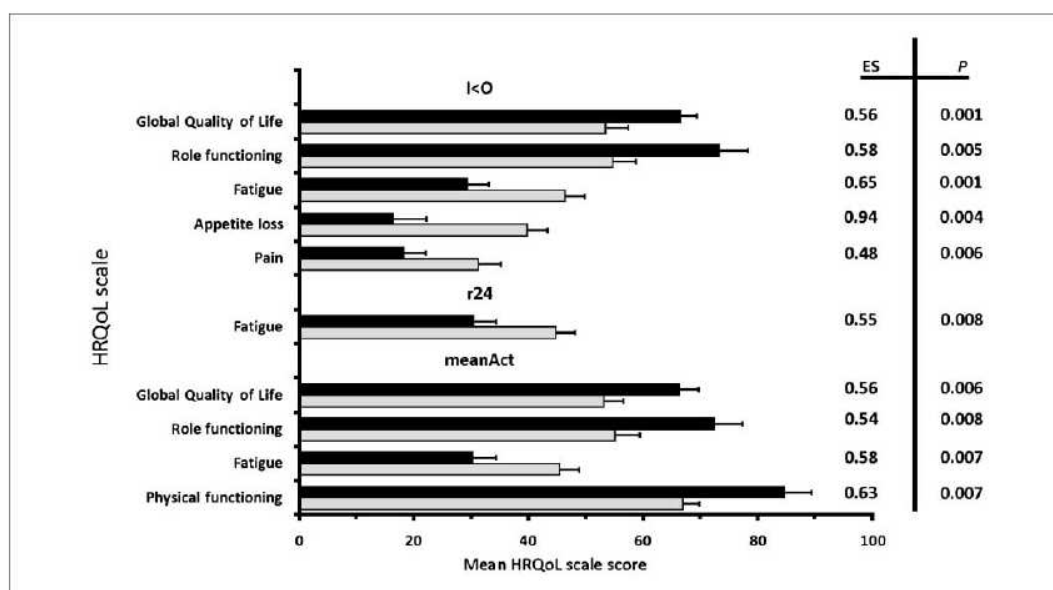


Figure 2. Difference in mean \pm SE HRQoL scores between two subgroups of patients classified by CircAct parameters above median (black columns) versus below the median (gray columns) where the clinical relevance (effect size ≥ 0.4) was statistically significant ($P < 0.01$). Black boxes, CircAct parameters above median: I<O $\geq 97.0\%$ ($n = 49$), $r24 \geq 0.38$ ($n = 47$), and meanAct ≥ 106 counts/min ($n = 49$). Gray boxes, CircAct parameters below median: I<O $< 97.0\%$ ($n = 47$), $r24 < 0.38$ ($n = 49$), and meanAct < 106 counts/min ($n = 47$).

main prognostic factors in metastatic colorectal cancer (31, 35) as well as age, gender, body mass index, institution, prior adjuvant chemotherapy, primary tumor site, and treatment arm in a Cox's multivariate regression analysis (hazard ratio, 0.94; 95% confidence interval, 0.92-0.97; $P < 0.0001$). Of those factors, the number of metastatic sites (1 versus >1) and the WBC count (<10 versus $\geq 10 \times 10^9$ cells/L) were significantly related to survival ($P = 0.041$ and 0.01, respectively). The bootstrap analysis showed percentages of inclusion in the multiple regression models of 89% for I<O, 74% for PS, and $<60\%$ for other parameters. I<O remained independently related to overall survival (hazard ratio, 0.93; 95% confidence interval, 0.90-0.96; $P < 0.0001$) after excluding the 23 patients who had received prior adjuvant chemotherapy. Conversely, $r24$ did not display an independent prognostic value in a Cox's multivariate regression analysis ($P = 0.13$) after adjustment for the aforementioned factors.

Discussion

The focus of this study was to correlate the circadian rest-activity rhythms of patients with advanced colorectal cancer with quality of life and survival. In this international study involving 130 patients with metastatic colorectal cancer, wrist actigraphy monitoring provided three objective parameters that were selected based on their clinical relevance in a previous study (31). The correlation between CircAct and selected HRQoL scales was confirmed as well as the independent prognostic value of CircAct for survival.

Both $r24$ and I<O, which quantify different aspects of CircAct, were strongly correlated with each other yet more weakly so with meanAct. The average level of activity, which does not account for

any circadian rhythmicity, was not as clinically relevant, highlighting the clinical importance of circadian physiology as opposed to the mere count of physical activity. Nevertheless, meanAct correlated with the two rhythm parameters, suggesting that a dampened CircAct was associated with reduced average activity. These CircAct parameters do not constitute an objective evaluation of sleep. Other actigraphy parameters, not assessable in the current study, have been reported to correlate with subjective sleep perception as estimated with specific sleep questionnaires (14, 36, 37). A specific assessment of sleep quality and quantity of 95 cancer patients with 42 h continuous ambulatory polysomnography revealed disturbances in sleep and waking states maintenance, with features supporting a blunted sleep drive from the circadian timing system (38).

PS (WHO), a subjective estimate of physical performance, was significantly correlated with I<O and $r24$. Conversely, the extent of disease involvement did not seem to influence the rhythmic pattern of activity. Damped circadian rhythms have been described in cancer patients with no evidence of disease, for example, in the adjuvant setting (14, 36), suggesting that factors other than tumor burden can account for circadian disruption.

The rest-activity rhythm provides a window on the circadian timing system, which controls hormonal patterns, sleep, food intake, and other rhythmic behaviors (1-3). Disturbances of the circadian timing system are associated with a cluster of symptoms, including fatigue, anorexia, and sleep disturbances (16-18, 39, 40). Damped CircAct was associated with subjective impairment in the physical, role, and social functioning dimensions of HRQoL (with more fatigue, anorexia, pain, dyspnea, and insomnia) as well as with poor global quality of life. These correlations were barely altered by age, body mass index, or hemoglobin concentration

(Table 2), underpinning the hypothesis of an independent role of the circadian timing system on the well-being of patients. These findings further extended and strengthened the relevance of previous results from a single institution study (31, 32). The order of magnitude of the correlation coefficients between CircAct parameters and HRQoL scales ($r \leq 0.4$; Table 2) was comparable

with that reported in other studies correlating an objective and unidimensional biological parameter, such as hemoglobin, with the subjective measure of HRQoL (41, 42).

In the current study, CircAct parameters predicted neither for best objective tumor response nor for progression-free survival. In our previous study, the association between CircAct parameters

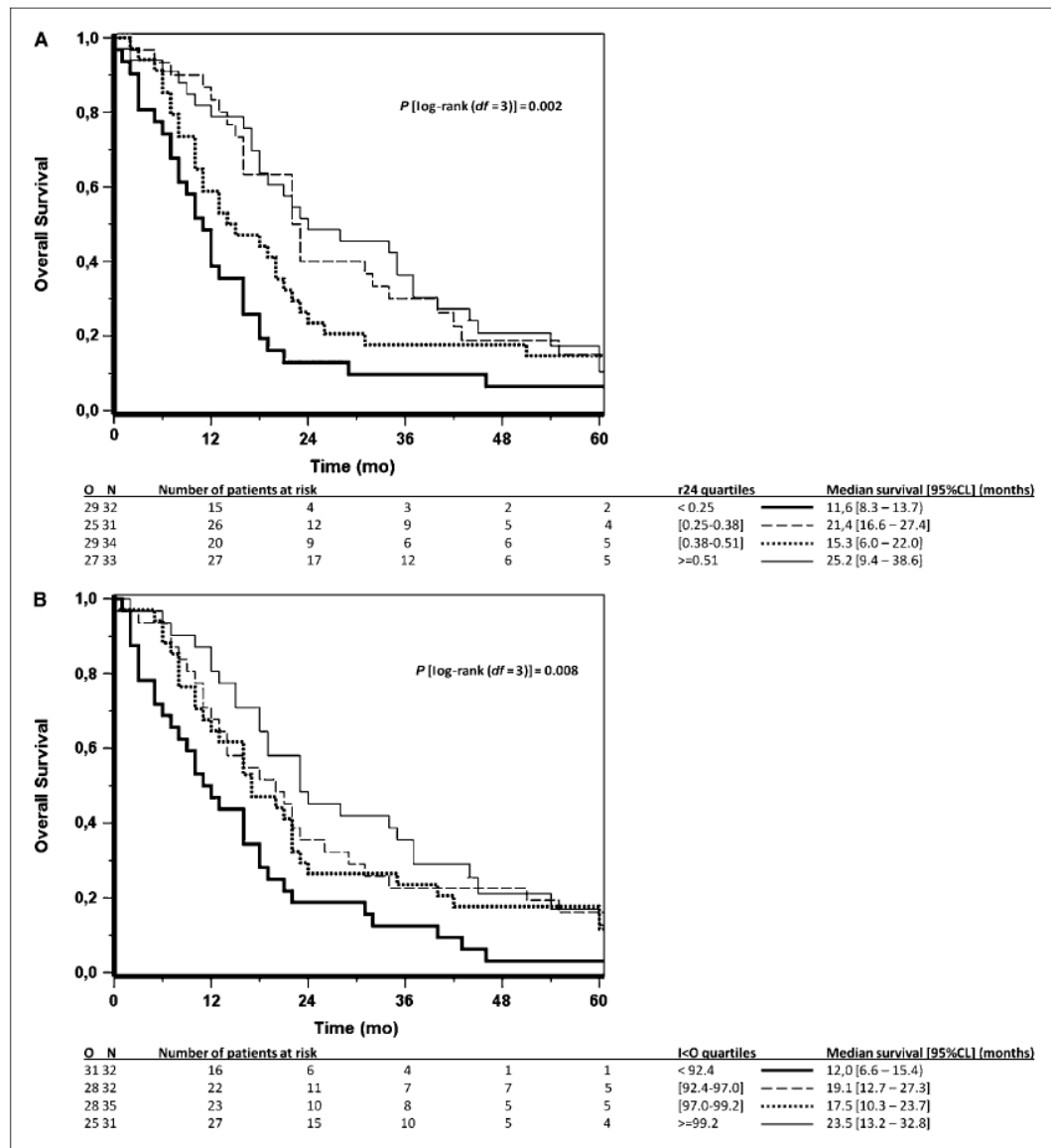


Figure 3. Kaplan-Meier survival curves according to r24 (A) and l<O (B) split by the quartiles of their distribution. Thick solid line, first quartile; thin dashed line, second quartile; thick dotted line, third quartile; thin solid line, fourth quartile. Log-rank test ($df = 3$): $P = 0.002$ for r24 (A) and $P = 0.008$ for l<O (B).

and progression-free survival was not investigated, but I-O was found to independently predict for tumor response (31). This discrepancy could relate to differences between the two studies regarding the proportion of chemotherapy-naïve patients (all versus 39%), the subsequent administration of chronomodulated chemotherapy (48% versus all), and the rate of best objective response (52% versus 35%, respectively; refs. 31, 33).

The peripheral tissue clocks determine the temporal patterns of drug cytotoxicity (1, 43, 44). The CircAct is a marker of the output from the central circadian pacemaker and does not reflect the rhythmicity of molecular clocks in peripheral tissues. Thus, alterations of host CircAct may only marginally affect the time-dependent antitumor activity and normal tissue toxicity. This could account for the lack of correlation between CircAct parameters and tumor response, progression-free survival, or toxicity.

In the present study, I-O was confirmed as an independent prognostic factor for overall survival. The lesser relevance of r24 compared with I-O for HRQoL and survival could indicate that the regular sequence of daytime highly active spans and restful nighttime sleep spans (as measured with I-O) rather than the consistent reproducibility of the activity pattern over exactly 24 h (as estimated by the r24) better reflect the functional status of the circadian timing system.

The trend in overall survival, which differentiated the quartiles of I-O, persisted with time elapsing after CircAct assessment. This observation indicated that the relationship between CircAct and overall survival was independent from immediate premortality disruption in circadian rest-activity cycles (Fig. 3B).

Social synchronization represents an essential time cue for the entrainment of human circadian rhythms (4, 45). A significant correlation between the social functioning dimension of HRQoL and I-O was revealed in both our previous and current studies (32). The independent prognostic value of social functioning for survival has been separately shown in all patients enrolled on the EORTC

05963 trial that completed the EORTC Quality of Life Questionnaire-C30 questionnaire at baseline (46), confirming previous results (47). Impaired social functioning, perceived as interference with family life and social activities, could reflect the patient's self-sensation of cancer-related physiologic disturbances.

Taken together, these data corroborate the hypothesis that the circadian rest-activity rhythm, measured with wrist actigraphy, correlates with several symptoms and domains of well-being as well as with the survival of patients with metastatic colorectal cancer.

The circadian timing system constitutes a novel potential therapeutic target for future multidisciplinary research efforts (44, 48, 49). Interventions that normalize circadian timing system dysfunction should be studied with the aim of relieving symptoms, improving HRQoL and prolonging survival in cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Association d'une disruption circadienne avec la fatigue et l'anorexie chez les patients atteints de cancer avancé: implication pour une approche thérapeutique innovante.

Innominato PF, Mormont MC, Rich TA, Waterhouse J, Lévi FA, Bjarnason GA.

Les patients atteints de cancer présentent fréquemment une disruption du système circadien, mise en évidence par le suivi de rythmes marqueurs. L'enregistrement non invasif du rythme d'activité-repos par un actimètre de poignet a été couramment utilisé à cet effet et permet une estimation robuste de la coordination circadienne. Les altérations du rythme circadien d'activité-repos sont significativement et indépendamment associées à la sévérité de la fatigue et de l'anorexie chez les patients atteints de cancer colorectal métastatique. Des taux de cytokines pro-inflammatoires élevées pourraient en partie expliquer cette perturbation du rythme circadien ainsi que les symptômes systémiques associés. Ici, les auteurs présentent et discutent les données soutenant l'hypothèse d'une association entre perturbation du rythme circadien et fatigue et anorexie. Leur tour ces symptômes modifient et atténuent la coordination circadienne, créant un cercle vicieux conduisant à la détérioration du système circadien. Cette hypothèse ouvre la voie à des approches thérapeutiques innovantes ciblant le système circadien afin de diminuer les symptômes systémiques induits par le cancer et certains traitements anticancéreux.

Circadian Disruption, Fatigue, and Anorexia Clustering in Advanced Cancer Patients: Implications for Innovative Therapeutic Approaches

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Abstract

A disruption of the circadian timing system, as identified by monitoring of marker biorhythms, is common in cancer patients. The recording of the rest–activity rhythm with a wrist actigraph has been commonly used. This noninvasive monitoring allows a robust estimation of circadian disruption. The authors have previously found that altered patterns of circadian rest–activity rhythms are significantly and independently associated with the severity of fatigue and anorexia in patients with metastatic colorectal cancer. Elevated proinflammatory cytokines could partly account for this circadian disruption and its associated constitutional symptoms. Here, the authors present and discuss the data supporting the hypothesis that circadian disruption is often associated with fatigue and anorexia, which in turn further alter and dampen circadian synchronization, thus, creating a vicious cycle. This body of evidence paves the path for innovative therapeutic approaches targeting the circadian timing system in an effort to diminish constitutional symptoms induced by cancer and some anticancer treatments.

Keywords

circadian, actigraphy, cancer, fatigue, anorexia

Circadian Disruption in Cancer Patients

The circadian timing system is a hierarchical network that generates endogenous rhythmic oscillations in various physiological parameters along the 24-hour span.^{1–3} Its correct functioning allows the organism to better adapt to and anticipate rhythmic variations of the external environment. A central master pacemaker, the suprachiasmatic nucleus in the hypothalamus, temporally influences several aspects of the host physiology, including locomotor activity, sleep–wake cycles, and hunger and hormonal rhythms.^{1–3} Disruption of many circadian marker rhythms has been documented in cancer patients.^{4,7} Depending on the monitoring method used, circadian disruption can result in amplitude dampening, abolition of rhythmic patterns, a shift of phase, or shortening or lengthening of the period.^{6,7} The vast majority of useful data in cancer patients has been obtained with minimally invasive or noninvasive methods of assessment of a marker biorhythm, such as salivary cortisol and locomotor activity.

Saliva samples have been obtained for at least 2 consecutive days at 3 time points—awakening (ie, around 08:00 hours), afternoon (ie, 16:00 hours) and late evening (ie, 23:00)—to obtain a daily pattern that under normal circumstances shows a morning peak with a steep slope along the day, with a trough in the night, before a new peak arises the following morning (Figure 1). In patients with advanced colorectal cancer, erratic, flat, or inverted

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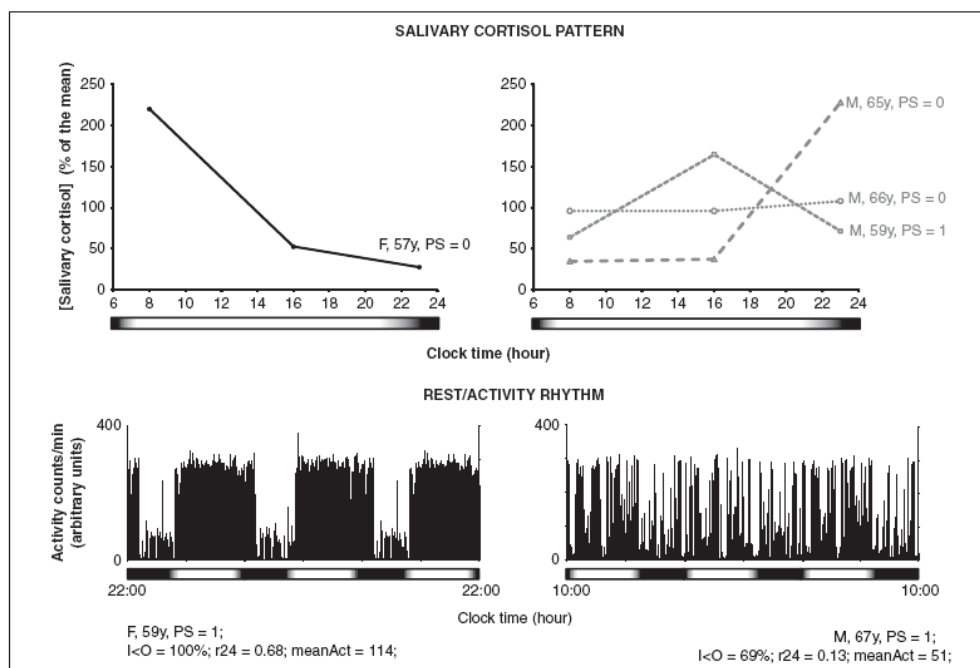


Figure 1. Top panels: examples of daily pattern of salivary cortisol in patients with metastatic colorectal cancer. Left panel: normal diurnal steep slope with a morning peak; right panels: abnormal patterns, with no identifiable peak at the 3 sampling times (circles), with afternoon (squares) or with night peak (diamonds). Bottom panels: examples of circadian activity rhythms from wrist actigraphy monitoring for 72 continuous hours. Left panel: marked rhythm with good reproducibility and large difference between the active and the rest phases, that is, with high interday stability (high r_{24}) and high intraday variability (high $I<O$); right panel: disrupted rhythm with low difference between day and night (low $I<O$) and with little reproducibility day by day (low r_{24})

patterns have been described (Figure 1). Similar observations have been reported for metastatic breast cancer patients.⁸

Locomotor activity, on the other hand, can be monitored for longer time frames with a wrist accelerometer, called an actigraph. This device continuously and noninvasively records the number of wrist accelerations per time unit (usually 1 minute). The plot of the wrist accelerations per minute along the monitoring time frame provides a pattern that normally shows high counts during the daytime and low counts during the rest phase at night (ie, a high intraday variability), with a robust reproducibility along the following days (ie, with a high interday stability; Figure 1). Disruption of the circadian pattern can be visually observed and objectively quantified with robust and validated parameters (eg, r_{24} , $I<O$, and mean activity).⁹⁻¹⁴

Wrist actigraphy recordings provide a noninvasive and reliable marker rhythm of the functioning of the circadian system that affects the temporal patterns of locomotor activity^{15,16} and organizes some other physiological rhythms.¹⁻³ Given the heterogeneity in the methodological procedures used to assess circadian function in cancer patients in published reports, it is difficult to precisely estimate the prevalence of circadian disruption, but it likely affects at least 25% to 50% of, if not most, patients with metastatic disease.¹⁷ Moreover, within the same patient, when several marker rhythms have been examined, an alteration in some, but not all of them, has often been observed, suggesting that each of the marker rhythms can reflect different compartments of the circadian timing system.⁶ Indeed, we have concomitantly monitored wrist actigraphy for 72 hours and measured plasma cortisol at

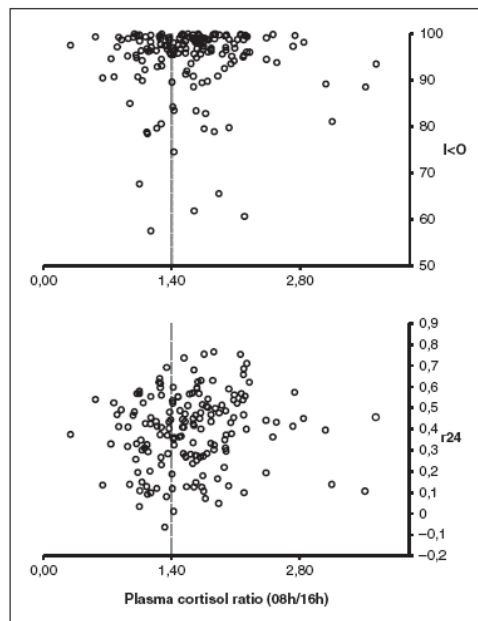


Figure 2. Distribution of the 165 patients with metastatic colorectal cancer according to their plasma cortisol ratio between 08:00 hours and 16:00 hours (normal value ≥ 1.4 ; X axis) and their wrist actigraphy parameters I<O (top panel) and r24 (bottom panel; Y axes). No correlation can be identified: $r = 0.05$, $P = .54$ for I<O, and $r = 0.14$, $P = .07$ for r24 (Spearman's rank correlations)

08:00 hours and at 16:00 hours for 2 consecutive days in 165 patients with metastatic colorectal cancer.¹⁴ The ratio between the value of plasma cortisol at 08:00 hours and 16:00 hours provides a reliable estimate of the circadian cortisol pattern.¹⁸ We have not observed any clear correlation between circadian rest-activity parameters and plasma cortisol ratio in this rather large group of patients (Figure 2). This suggests that the phenotype of circadian disruption may be a result of disruptions in different aspects of circadian physiology in each individual patient or that much more frequent biochemical measurement of cortisol and other markers over much longer time spans is essential for reliable correlations with minute-by-minute measured circadian activity patterns.

Fatigue in Cancer Patients

Cancer-related fatigue, defined by the National Comprehensive Cancer Network (NCCN) Fatigue Guidelines

Committee as "an unusual, persistent, subjective sense of tiredness related to cancer or cancer treatment that interferes with usual functioning," (p. 5) is the most prevalent symptom experienced by cancer patients.¹⁹ It is estimated that 95% of patients who are scheduled to receive chemotherapy or radiotherapy experience some degree of fatigue during their treatment, with almost every study reporting an incidence of at least 70%.¹⁹⁻²² Not only is fatigue highly prevalent, but it is also reported to be the most distressing symptom, affecting the patient's quality of life and daily activities negatively.¹⁹⁻²¹ Given this wide prevalence among cancer patients, and even cancer survivors several years after complete remission,^{23,24} it is clear that the underlying mechanisms may deeply involve various components of human physiology. Thus, possible causes of fatigue can involve the periphery (eg, neuromuscular junctions and proprioceptive fibers), the central nervous system (eg, neuromediator imbalance, inflammatory cytokines, hormonal dysfunction, autonomic deregulation, and circadian disruption), and comorbid conditions, such as anemia, cachexia, and depression. In this review, we focus on the association of fatigue and circadian disruption because circadian rhythms temporally control several aspects of human physiology linked to fatigue, such as neuromuscular and cognitive performance, brain neuronal activity, hormones, and inflammation-immunity mediators.^{5,25-27}

Anorexia in Cancer Patients

Similarly to fatigue, appetite loss is a frequent complaint of cancer patients, with an incidence of the order of 50%.²² Peripheral problems involving gastroenteric peristalsis, abdominal bloating, and chemosensory dysfunction can affect a patient's appetite. Central mechanisms accounting for dwindling appetite include fatigue, inflammatory signals mediated by cytokines, and hormonal, autonomic, and neuromediator imbalances.²⁸⁻³⁰ Other comorbidities, including depressive symptoms, psychological distress, social withdrawal, and nausea, can further contribute to this problem. Hunger displays a strong circadian rhythm.³¹⁻³³ Jet lag and shift work, two conditions affecting circadian internal synchronization, are also associated with appetite loss.^{25,34-38} It is therefore possible that circadian disruption contributes to anorexia in cancer patients.

Symptom Clusters in Cancer Patients

The frequent occurrence of multiple, simultaneous subjective complaints by individual cancer patients implies a shared etiology. This observation has led to the definition of symptom clusters as groupings of at least 2 related, co-occurring symptoms that reproducibly coexist in the same

Table 1. Clinical Studies Correlating Wrist Actigraphy Parameters (Estimating Activity, Sleep, and/or Circadian Rhythm) With Subjective Fatigue in Cancer Patients

Cancer Setting	Number of Patients	Fatigue Questionnaire Used	Objective Actigraphy Parameter(s) Correlating With Fatigue	References
Breast cancer survivors	200	FACT-F, BFS, FCS, WAS, EORTC QLQ-C30	Sleep	Alexander et al ⁴⁹
Breast cancer	72	PFS	Activity and sleep	Berger ⁵¹ and Berger and Farr ⁵²
	14	PFS	Activity and sleep	Berger and Higginbotham ⁵⁴
	78	MAF, FSCL	Circadian, activity and sleep	Roscoe et al ⁶⁰
	85	MFSI-SF	None	Ancoli-Israel et al ⁵⁰
	16	FVAS	Sleep	Kuo et al ⁵⁷
	130	PFS	Circadian, activity and sleep	Berger et al ⁵³
Colorectal cancer	190	PFS	Circadian	Berger et al ⁵⁵
	192	EORTC QLQ-C30	Circadian and activity	Mormont et al ¹⁴
Various cancers	96	EORTC QLQ-C30	Circadian and activity	Mormont and Waterhouse ⁵⁹
	24	LFS	Sleep	Innominato et al ¹³
	7	LAS-F	Activity	Miaskowski and Lee ⁵⁸
	25	EORTC QLQ-C30	None	Sarna and Conde ⁶¹ Fernandes et al ⁵⁶

NOTES: FACT-F, Functional Assessment of Cancer Therapy-Fatigue; BFS, Bidimensional Fatigue Scale; FCS, Fatigue Catastrophizing Scale; WAS, Work and Social Adjustment Scale; EORTC QLQ-C30, European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire C30; PFS, Piper Fatigue Scale; LFS, Lee Fatigue Scale; LAS-F, Linear Analogue Scale-Fatigue; MAF, Multidimensional Assessment of Fatigue; FSCL, Fatigue Symptom Checklist; MFSI-SF, Multidimensional Fatigue Symptom Inventory-Short Form; FVAS, Fatigue Visual Analogue Scale.

patient and are relatively independent of other symptom groups.³⁹ The concept of symptom clusters has been recognized in symptom management research and has important therapeutic implications for cancer patients.⁴⁰⁻⁴³ In studies assessing symptom clusters, fatigue (asthenia, weakness, weariness, lethargy, drowsiness, etc) and anorexia (lack of appetite, early satiety) frequently cluster together.^{22,40-42,44,45} Moreover, several studies have further associated this symptom cluster of fatigue and anorexia with sleep difficulties and neuropsychological distress—two disturbances clearly linked to disruption of the circadian timing system.^{38,42,46-48}

Correlations Between Objective Circadian Disruption and Subjective Symptoms

Data on wrist actigraphy have been published for 2675 adult oncology patients. The parameters derived from the activity data vary somewhat based on the clinical end point of each study. Actigraphy monitoring has been used to measure the overall locomotor activity, the circadian pattern of rest-activity rhythm, and to evaluate sleep quality and quantity.⁹ In some studies, actigraphy data have been correlated with questionnaires that attempt to measure symptoms, quality of life, and sleep. Here, we will focus on the reports of fatigue and/or anorexia.

Table 1 summarizes the data for 1129 cancer patients for whom actigraphy data were correlated with data from fatigue questionnaires.^{13,14,49-61} In 2 out of 13 studies, no significant correlation between actigraphy parameters and fatigue was described.^{56,61} In the remaining 11 studies, performed in patients with different types of cancer, using different actigraphy devices and different fatigue scales, the degree of fatigue was found to be associated with circadian disruption, poor sleep, or lower daytime physical activity.^{13,14,49-55,57-60} Current data thus support the hypothesis that a disturbance in circadian function, as estimated by a locomotor activity biomarker, is associated with increased fatigue. The heterogeneity of the methodological approaches and reporting procedures used worldwide calls for collaborative efforts, as has been pointed out by other authors.⁹

The association between the severity of abnormalities in circadian actigraphy data and anorexia in cancer patients is limited to fewer studies. Daily physical activity was correlated positively with serum albumin and negatively with weight loss (3 objective measures) in 53 patients with advanced cancers.⁶² In 2 independent cohorts of 192 and 96 metastatic colorectal cancer patients, robust and reproducible circadian activity patterns (objectively evaluated with wrist actigraphy parameters) were correlated with less severe anorexia (subjective measure).^{13,14} In these 2 cohorts, those parameters were also negatively correlated with fatigue (subjective measure).^{13,14}

Table 2. Distribution of the 251 Advanced Colorectal Cancer Patients According to the Quartiles of Their Subjectively Rated Fatigue and Anorexia (EORTC QLQ-C30 Questionnaire)^a

			Fatigue Quartiles			
			I	II	III	IV
Scale values			0-11.1	22.2-44.4	55.6-77.8	88.9-100
Anorexia quartiles	I	0	19.5	27.1	8.4	1.6
	II	33.3	2.0	7.6	11.2	1.6
	III	66.7	0	1.6	8.4	2.8
	IV	100	0.4	0.8	3.2	4.0

NOTES: EORTC QLQ-C30, European Organisation for Research and Treatment of Cancer Quality of life Questionnaire C30.

^aSymptom scales can range between 0 and 100. The numbers in the cells indicate the percentages of the whole population with the corresponding scale values of fatigue and anorexia. We have highlighted in gray the subgroup of patients (22.7%) with a clinically relevant constitutional symptom, that is, with either fatigue or anorexia, rated in the highest (fourth) quartile, or with both fatigue and anorexia, rated in the third quartile.

Here, we report for the first time the results of the combined analysis of these 2 data sets.^{13,14} This data set consists of 251 patients with metastatic colorectal cancer, who each wore the actigraph for 72 consecutive hours and who also completed the EORTC QLQ-C30 (European Organisation for Research and Treatment of Cancer Quality of life Questionnaire C30) questionnaire⁶³ to estimate their subjective fatigue and anorexia. Actigraphy monitoring provided 2 parameters, which estimated the robustness and reproducibility of the circadian activity pattern (r_{24}) and also the intraday variability between the active and the rest phases over 24 hours ($I < O$), as well as the mere count of average wrist movements per day (meanAct).^{13,14} The severity of fatigue and anorexia was transformed into a scale ranging from 0 (no symptom) to 100 (highest self-perception of the symptom).⁶³ We divided our population into quartiles according to the severity of fatigue and anorexia (Table 2). We considered that a patient experienced a clinically significant distress from the symptom if he or she rated either fatigue or anorexia in the highest quartile or if he or she rated both fatigue and anorexia in the third quartile or higher (Table 2). Using this categorization, we defined a subgroup of 57 patients (22.7%; highlighted in gray in Table 2) with a high degree of fatigue and/or anorexia and another subgroup of 194 patients (77.3%) with none or with a much lower degree of these systemic symptoms. The subgroup of patients with distressing fatigue and anorexia symptoms displayed significantly more disrupted circadian activity and sleep, as estimated by both $I < O$ ($P < .0001$) and r_{24} ($P < .0001$), as well as significantly lower average daily physical activity ($P < .0001$; Figure 3). Moreover, we observed a significant clustering of high fatigue scores, high anorexia scores, and low actigraphy parameters within the same patient group (Figure 4). This clustering of circadian disruption with more

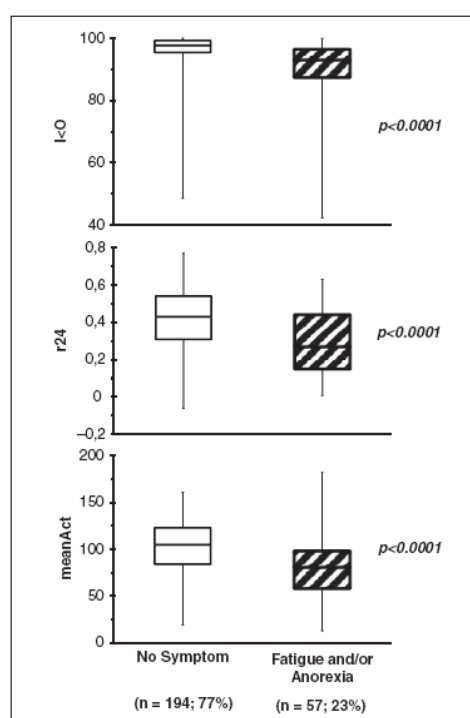


Figure 3. Distribution of the actigraphy parameters $I < O$ (upper panel), r_{24} (middle panel) and meanAct (bottom panel) according to the absence (white boxes) or presence (slanted lines) of clinically significant fatigue and/or anorexia: the box shows the median (middle line) and the first and third quartiles; the vertical bars indicate the range (Mann-Whitney U test)

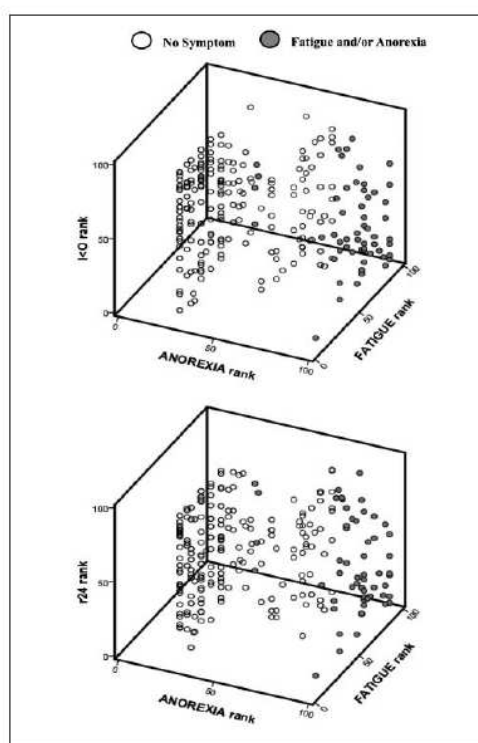


Figure 4. Distribution of the 251 patients with metastatic colorectal cancer according to the fractional rank of the values of self-rated fatigue (right axis) and anorexia (left axis), and according to the fractional rank of the objectively calculated actigraphy parameter (vertical axis) I<O (top panel) and r24 (bottom panel): In gray, patients with clinically significant fatigue and/or anorexia; in white, patients without relevant systemic symptoms

severe fatigue and anorexia suggests common etiological mechanisms.⁶⁴

Proinflammatory cytokines, produced in the periphery, can signal the central nervous system and may be involved in the etiology of symptom clusters in cancer patients.^{45,65-71} The term *cytokine-induced sickness behavior* has been coined and includes mainly fatigue and anorexia, together with a profound alteration of the usual daily rhythmic patterns of locomotor activity, sleep, and core body temperature.^{45,64,66,69,71-75} We have shown that patients with

metastatic colorectal cancer displaying disruption of their circadian rest-activity pattern have significantly higher circulating levels of proinflammatory cytokines TGF- α (tumor growth factor α), TNF- α (tumor necrosis factor α), and IL-6 (interleukin 6).⁷⁶ In all, 40 out of 80 patients studied had elevated levels of TGF- α (greater than the median value of 1.35 pg/mL) and concurrently showed significantly more pronounced circadian disruption (ie, lower values of I<O and r24) and more severe fatigue and anorexia (Figure 5).

Fatigue and/or anorexia can have a detrimental effect on the physiological synchronization of the circadian timing system. External cues, such as social routine and timing of food intake, normally help synchronize and entrain the circadian timing system.⁷⁷⁻⁸¹ Impaired physical function with resulting social withdrawal, anorexia, and less-consistent meal timing can be expected to blunt the synchronizing effects of these circadian cues (Figure 6).

Anorexia and fatigue are not only the most prevalent cancer-related complaints but are also the most common adverse effects of basically all chemotherapy agents.^{22,24,69} Fatigue and anorexia (and subsequent objective weight loss) form a constitutional cluster of toxicities induced by chemotherapy.^{82,83} An increase in proinflammatory cytokines has been described following chemotherapy administration,^{45,69,84-90} possibly accounting for treatment-related symptoms, including fatigue and anorexia.^{86,91} Furthermore, actigraphy monitoring has shown that chemotherapy can induce abnormal sleep-activity rhythms and sleep disruption in cancer patients.⁹²⁻⁹⁵

Potential Novel Therapeutic Approaches

Disrupted circadian function may be responsible for numerous symptoms in cancer patients.^{25,34-36,38,96-99} The body of data reviewed above shows that circadian rhythm disruption is associated with fatigue and anorexia, which in turn can exacerbate circadian disruption, thus, contributing to the vicious circle of deterioration often seen in cancer patients.

Currently, there are no effective therapeutic options for preventing or treating cancer-associated fatigue and anorexia.^{28,29,100,101} Currently available drugs used to palliate these symptoms act on one mediator or one causative mechanism.^{28,41,42,100,101} Therapeutic interventions targeting the circadian timing system have the potential advantage of acting concurrently on multiple aspects of human physiology¹³⁻⁹⁷ and represent an innovative opportunity to improve symptom management in cancer patients.^{13,65,102-107}

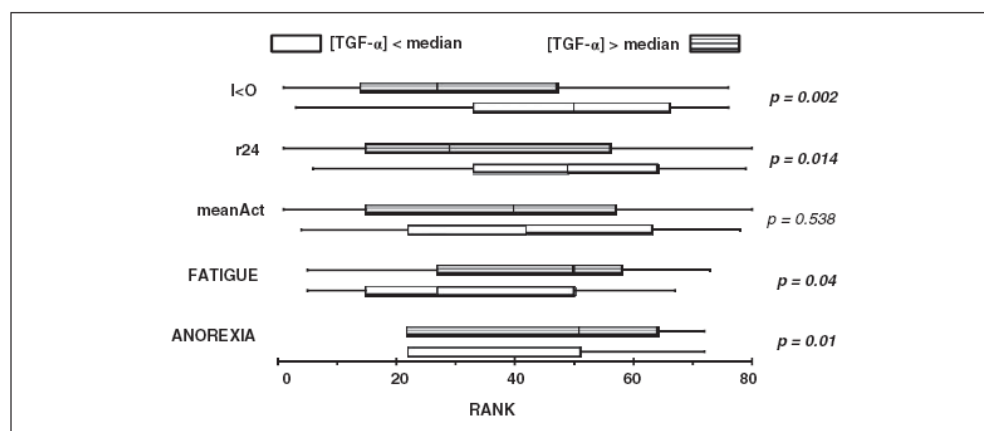


Figure 5. Boxplots illustrating the distribution of the ranks of actigraphy parameters I<O, r24, meanAct, and of the ranks of fatigue and anorexia according to the serum levels of TGF (tumor growth factor)-α below the median of 1.35 pg/mL (n = 40; white) or above the median (n = 40; horizontal stripes). Boxes represent the median and the first and third quartiles. Horizontal bars represent the range (Mann-Whitney U test)

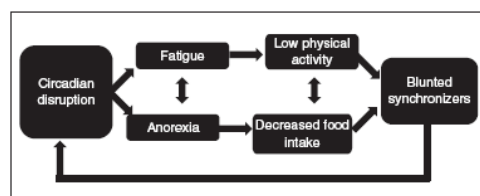


Figure 6. Schematic representation of the bilateral interactions between disruption of the circadian cycle with fatigue and anorexia: alteration of the function of the circadian timing system can cause these systemic symptoms, which induce a blunting of their synchronizing effects on the circadian timing system, thus, aggravating its disruption

Declaration of Conflicting Interests

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V.3. Modifications du rythme circadien provoqués par la chimiothérapie

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Rythmes de la température cutanée superficielle comme biomarqueur potentiel pour guider la personnalisation de la chronothérapie des cancers.

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La chronothérapie implique l'administration des traitements en fonction des rythmes circadiens. Le choix du stade circadien d'administration des médicaments anticancéreux a permis d'en améliorer la tolérance jusqu'à cinq fois et l'efficacité jusqu'au double dans des études expérimentales et cliniques. Cependant, les composants physiologiques et moléculaires du système circadien, ainsi que le sexe, influencent l'activité d'un schéma chronothérapeutique standardisé. En outre, une thérapie administrée au mauvais moment ou une dose excessive du médicament perturbe le système circadien. Par conséquent, une approche non-invasive est nécessaire pour détecter et suivre avec précision les rythmes circadiens et réaliser ainsi une évaluation dynamique du système circadien afin de personnaliser le schéma d'administration chronomodulée chez les patients cancéreux. Puisque la température corporelle centrale du corps est un biomarqueur circadien robuste, nous avons enregistré en continu la température cutanée en plusieurs endroits du thorax et du dos chez des sujets sains et des patients atteints de cancer. Une variabilité de la phase circadienne existait selon l'emplacement des « patches » thermiques, chez les individus sains ou malades au cours des 2 à 6 jours d'enregistrement. Cette étude démontre la nécessité d'enregistrer la température cutanée en plusieurs emplacements afin de définir précisément la phase circadienne. De plus, nous avons observé que les régions cutanées identifiées par imagerie infrarouge comme relativement froides présentaient les plus amples

variations thermiques sur 24 heures. L'existence d'altérations du rythme thermique cutané au cours d'une chronochimiothérapie intensive démontre la nécessité d'une évaluation continue du système circadien et d'une personnalisation des schémas chronothérapeutiques.

Skin surface temperature rhythms as potential circadian biomarkers for personalized chronotherapeutics in cancer patients

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Chronotherapeutics involve the administration of treatments according to circadian rhythms. Circadian timing of anti-cancer medications has been shown to improve treatment tolerability up to fivefold and double efficacy in experimental and clinical studies. However, the physiological and the molecular components of the circadian timing system (CTS), as well as gender, critically affect the success of a standardized chronotherapeutic schedule. In addition, a wrongly timed therapy or an excessive drug dose disrupts the CTS. Therefore, a non-invasive approach to accurately detect and monitor circadian rhythms is needed for a dynamic assessment of the CTS in order to personalize chronomodulated drug delivery schedule in cancer patients. Since core body temperature is a robust circadian biomarker, we recorded temperature at multiple locations on the skin of the upper chest and back of controls and cancer patients continuously. Variability in the circadian phase existed among patch locations in individual subjects over the course of 2–6 days, demonstrating the need to monitor multiple skin temperature locations to determine the precise circadian phase. Additionally, we observed that locations identified by infrared imaging as relatively *cool* had the largest 24 h temperature variations. Disruptions in skin temperature rhythms during treatment were found, pointing to the need to continually assess circadian timing and personalize chronotherapeutic schedules.

Keywords: chronotherapeutics; circadian rhythm; biomarker; body temperature; cancer; personalized medicine

1. INTRODUCTION

Circadian rhythms (with approximately a 24 h period) have been shown for most biological variables in many living organisms, including cyanobacteria, plants, flies, rodents and humans [1,2]. Rhythms on other time scales also characterize biological functions, such as ultradian hourly rhythms in pituitary hormonal secretions or NF-κB cellular signalling pathways, and yearly rhythms in the reproductive behaviour of mammals [3–6]. Circadian rhythms are especially relevant

for anti-cancer therapy, since they regulate drug metabolism and gate cell division over the 24 h [5,7]. Circadian rhythms are generated within each cell by molecular clocks, consisting of interwoven transcription/translation feedback loops involving 15 ‘clock’ genes [4]. The molecular clocks are coordinated during the 24 h by an array of physiological rhythms, which are generated by the suprachiasmatic nuclei (SCN). This circadian pacemaker, located in the hypothalamus, receives daily inputs from environmental cycles, and generates rhythmic physiological outputs, such as rest-activity, body temperature and hormonal secretions [5]. The circadian timing system (CTS) assembles these different components and regulates bodily functions over the 24 h. As a result, the CTS

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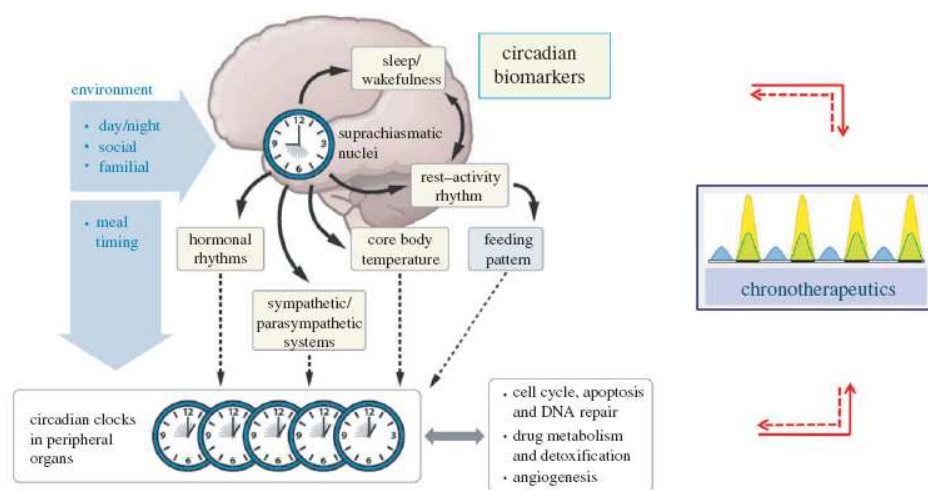
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Figure 1. Schematic of the circadian timing system (CTS). The CTS is composed of a hypothalamic pacemaker, the suprachiasmatic nuclei SCN, an array of SCN-generated circadian physiology outputs, and molecular clocks in the cells of peripheral tissues. Molecular clocks rhythmically control xenobiotic metabolism and detoxification, cell cycle, apoptosis, DNA repair and angiogenesis over a 24 h period. The CTS is synchronized with time cues provided by light–dark cycles and other environmental factors. Circadian physiology outputs can also serve as CTS biomarkers. Chronotherapeutics aim at improving chemotherapy tolerability and efficacy through the adjustment of drug delivery to the CTS, which can in turn be influenced by the treatment regimen (Reproduced with permission from *Annu. Rev. Pharmacol. Toxicol.* 50. Copyright © 2010 by Annual Reviews; <http://www.annualreviews.org>).

determines optimal times of day or night when anti-cancer medications are best tolerated and most effective. Cancer chronotherapeutics integrate the CTS control of biological functions into the design of chronomodulated drug delivery patterns in order to enhance tolerability and/or efficacy (figure 1; [5]).

In mice or rats with similarly synchronized CTS, the tolerability of 40 anti-cancer drugs varied two- to several fold as a function of circadian timing [5]. Moreover, anti-tumour efficacy was usually largest following treatment near the time of best tolerability, as shown for 28 anti-cancer medications [5]. Mathematical models have pinpointed several differences between the biological dynamics in healthy tissues and those in cancer, which could account for this coincidence between chronotolerance and chronoef- ficacy. These include a differential in (i) circadian entrainment, which is effective in healthy cells, but often lacking in cancer cells, (ii) cell cycle phase variability, (iii) cell cycle length, and (iv) tumour cell growth rate [8–11].

Clinical trials have shown the relevance of chronomodulated infusions of chemotherapy in cancer patients. Results from preclinical and clinical studies determined a standardized chronomodulated delivery pattern for the anti-cancer drugs 5-fluorouracil–leucovorin (5-FU–LV) and oxaliplatin, with peak flow rates at 4.00 h for 5-FU–LV and at 16.00 h for oxaliplatin (chronoFLO) [5]. Such a chronotherapeutic schedule standardized for the entire patient population improved tolerability up to fivefold and nearly doubled anti-tumour efficacy as compared with both constant rate infusion and chronomodulated schedules with peak

times of drug delivery shifted by 9–12 h [7,12]. In a subsequent randomized trial, chronoFLO prolonged survival in men, but not in women suffering from meta- static colorectal cancer, as compared with conventional treatment delivery [13]. This finding has just been con- firmed in the first meta-analysis of three randomized trials involving 842 patients with metastatic colorectal cancer [14]. Moreover, while optimal circadian timing remained within a 6 h window in men, a larger inter- patient variability in optimal timing was apparent in women, demonstrating that a standardized chrono- modulated delivery schedule may not be appropriate for an entire patient population [7]. In addition, severe toxicities were encountered 25–50% more frequently in women as compared with men, irrespective of sched- uling [7,13]. These findings led us to hypothesize that excessive toxicity from chemotherapy suppresses the circadian mechanisms needed for chronotherapeutic optimization, and that this occurs more frequently in women than in men.

Indeed, circadian disruption resulted from wrongly timed or excessively dosed chemotherapy, as shown for 12 anti-cancer agents in experimental models [5]. Furthermore, circadian disruption accelerated cancer growth in experimental models, as well as in cancer patients [15–20]. Experimental studies provided consistent evidence that the reinforcement of the CTS through small molecules, such as seliciclib, or meal timing inhibited cancer growth [21,22]. Thus, circadian biomarkers are needed for tailoring cancer chrono- therapeutics to the dynamics of circadian organization of an individual patient in order to personalize the drug delivery schedules.

Appropriate circadian biomarkers should help identify the optimal endogenous timing of anti-cancer treatments in an individual patient, detect CTS alterations and guide adequate treatment adjustments. CTS disruption through ablation of the hypothalamic pacemaker, iterative alterations of the light–dark cycles, clock gene mutations or cancer chemotherapy result in rest–activity rhythm alterations, so that this rhythm has played a major role for circadian phenotyping [4]. Rest–activity rhythm has been monitored non-invasively using a wrist actigraph in large cohorts of cancer patients. Statistically significant relations were found between rest–activity rhythm parameters and patient symptoms and quality of life [18]. Most importantly, rest–activity parameters also displayed statistically significant and independent prognostic values for patient survival [19,23]. However, the square waveform of the rest–activity pattern makes it a poor biomarker for the circadian phase since it mostly provides information on start and termination times of rest and activity spans, with their respective variability in amount of activity.

Core body temperature is a circadian biomarker that appears to robustly reflect CTS pacemaker function and provide relevant information on phase and amplitude both in experimental models [5] and in humans [24–26]. Furthermore, the circadian rhythm in body temperature plays an important role in the coordination of molecular clocks, as well as clock-controlled pathways in experimental tumours [22,27]. Based on this knowledge, we hypothesize that temperature monitoring may be an appropriate biomarker for personalization of cancer chronotherapeutics. On the one hand, core body temperature cannot be easily monitored non-invasively or continuously in patients. On the other hand, skin temperature patterns can display variable links with core body temperature and sleep patterns according to site of measurement, levels of activity of the subject and environmental conditions [28,29]. In addition, there is typical heterogeneity in skin temperature within a given area, possibly in relation to different properties in temperature regulation, and the underlying microvasculature. We approached these problems through placing thermal sensing patches over locally warmer or cooler skin surface areas as identified with an infrared (IR) camera, in the upper thorax and back torso of each subject. In this pilot study, we provide the first analysis of skin temperature dynamics recorded at multiple sites in healthy individuals and in cancer patients undergoing cancer chronotherapy. We further show inter- and intra-individual circadian dynamics which support the future aim of using temperature as a biomarker to personalize chronotherapeutics.

2. SUBJECTS AND METHODS

2.1. Subjects and experimental design

The study involved five controls, three women and two men, aged 26–61 years, and four cancer patients, three men and one woman, aged 27–69 years. All subjects signed informed consent that explained the study, its

goals, procedures and possible drawbacks and expectations. Two of the control subjects had intercurrent minor health events, which were unrelated to the study and required medication intake. Three cancer patients had received extensive chemotherapy and iterative surgical procedures for metastatic colorectal cancer. Two of them were receiving chronomodulated chemotherapy during the study with irinotecan, 5-fluorouracil, leucovorin and oxaliplatin over 4 consecutive days (chronoIFLO4) combined with bevacizumab for one patient, and cetuximab for the other one [30,31]. One patient with metastatic renal cell carcinoma was taking a single daily oral dose of everolimus in the late evening and gliclazide in the morning, following prior failure of sunitinib (table 1). The five controls and two cancer patients underwent their ordinary daily activities during monitoring, which was not the case for the two hospitalized cancer patients receiving intensive chronotherapy. All subjects kept a precise diary of their daily activities, including times of awakening and retiring, meal times, times of bath or shower, times of going in or out and times and doses of medication intake.

2.1.1. Thermal measurements. Wireless skin surface temperature patches (Philips Respironics, MA, USA), with set sampling rates of 15 s, temperature resolution of 0.01°C and battery life of 240 h were placed on each subject's skin to monitor temperature continuously for 39–120 h. Each subject carried a VitalSense monitor (Philips Respironics) that was capable of monitoring up to 10 patches simultaneously, and the subjects were asked to keep the monitor within the 2 m reception range at all times.

Six or seven skin surface temperature patches were placed on the chest and upper back of each subject, according to IR imaging with an FLIR SC7700 camera (FLIR Systems ATS, France) with 0.015°C temperature resolution in the 3–5 µm wavelength (at 640 × 512 pixels per image). The warmest and coolest locations of both the chest and the upper back of each subject were marked with a surgical pen, and the thermal sensor of each patch was positioned directly at the mark (figure 2). Images were acquired before and immediately after patch placement as well as before and immediately after patch removal. Table 2 summarizes the patch placement and recording characteristics for healthy controls (HC) and patients (PAT).

All subjects also carried an iButton temperature sensor (Maxim Integrated Products, Inc., CA, USA) and were instructed to keep the sensor within 2 m of their body; this monitored ambient air temperature with a sampling rate of 1/60 to 1/600 s, set according to the duration of a subject's participation in the study.

2.1.2. Rest–activity measurements. The rest–activity pattern was monitored in all the subjects using a Mini-Motionlogger wristwatch accelerometer (Ambulatory Monitoring Inc., NY, USA). Activity data were recorded simultaneously using zero-crossing mode (ZCM) and proportional integrating measure (PIM) algorithms. ZCM counts the number of times within a set epoch (1 min) that the accelerometer changes

Table 1. Subject characteristics.

	sex	age (years)	height (cm)	weight (kg)	start date	end date	intercurrent diseases	ongoing treatment (s)	comment
HC 1	F	26	165	65	07 Mar 2010	09 Mar 2010	infectious (chronic) sinusitis, measles on second day	montelukast (Singulair) 10 mg d ⁻¹ at 20 h, desloratadine (Aerius) 5 mg d ⁻¹ at 20 h	—
HC 2	M	61	182	93	07 Mar 2010	10 Mar 2010	none	none	—
HC 3	F	51	162	60	09 Mar 2010	11 Mar 2010	fracture of the fifth metatarsus of the left foot in consolidation	sodic fondaparinux (Arixtra) 2.5 mg d ⁻¹ (subcutaneous) at 22.30 h	—
HC 4	F	35	157	59	11 Mar 2010	14 Mar 2010	none	none	—
HC 5	M	26	180	75	23 Mar 2010	28 Mar 2010	none	none	—
PAT 1	M	69	160	55	16 Mar 2010	18 Mar 2010	multiple metastases from colon cancer (liver, lung and lymph nodes)	none	last course of AVIFP ^a (16–20 Mar 2010)
PAT 2	M	55	178	67	16 Mar 2010	18 Mar 2010	multiple liver metastases from colon cancer	Erb + chronoFLO ^b	daily supportive medications
PAT 3	F	27	162	59	17 Mar 2010	22 Mar 2010	multiple liver, lung and bone metastases from colon cancer	Erb + chronoFLO ^c	daily supportive medications
PAT 4	M	68	170	82	22 Mar 2010	25 Mar 2010	brain, lung, adrenal, skin, pancreatic and lymph node metastases from renal cancer	everolimus (Afinitor) 10 mg at 23.00 h in the evening, glidazide (Diamicon) 80 mg at 09.00 h in the morning	stereotaxic radiotherapy on frontal and occipital metastases on 24 Feb 2010

^aBevacizumab (1 day infusion) + chronomodulated irinotecan-5-fluorouracil-folinic acid (3 days, infusion).^bCetuximab (1 day infusion) + chronomodulated 5-fluorouracil, folinic acid and oxaliplatin (3 days, infusion).^cCetuximab (1 day infusion) + chronomodulated irinotecan, 5-fluorouracil, folinic acid and oxaliplatin (3 days, infusion).

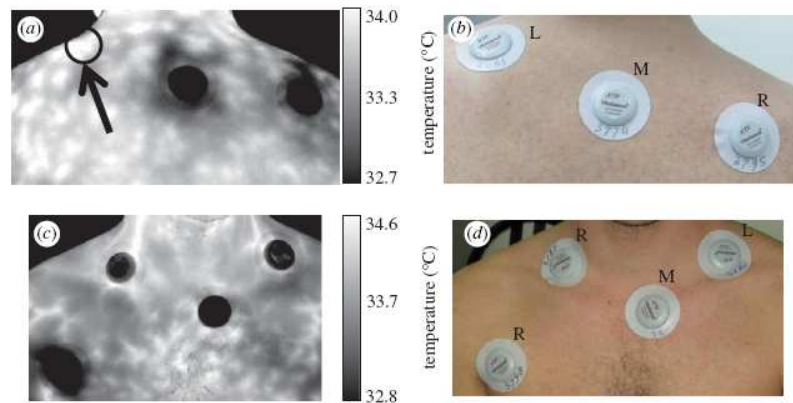


Figure 2. Example of patch placement on a control subject. Placement of patches was guided using infrared (IR) imaging to identify relatively warm and cool locations. Three patches were placed on the back of this subject, shown by the (a) infrared image and (b) visible image. In the visible image (b), superimposed 'R', 'M' and 'L' signify right, middle and left, respectively, and numbers on the patches represent a unique identification number for each patch. Lighter colours in the infrared images represent warmer temperatures. A warm location on the skin before patch placement is identified in (a) by the arrow and circle surrounding the region where the patch will be placed. (c) Infrared and (d) visible images of the subject's chest show four patches that are already placed, two on the right, one in the middle and one on the left side of the chest.

Table 2. Skin surface patch placement information. Number of patches analysed out of the total number of patches placed was determined based on the criteria in the text. Patches were placed on the chest or upper back, right, middle or left and on relatively warm or cool locations of the torso, as described in the text.

subject	recording length (h)	analysed-placed	chest-back	right-middle-left	warm-cool
HC 1	46.7	6-6	3-3	4-0-2	4-2
HC 2	39.3	1-6	3-3	4-0-2	4-2
HC 3	46.8	6-6	3-3	2-2-2	4-2
HC 4	72.3	4-5	3-2	2-1-2	3-2
HC 5	110.3	7-7	4-3	3-2-2	5-2
PAT 1	49.3	5-6	3-3	3-1-2	4-2
PAT 2	74.6	2-5	2-3	1-1-3	3-2
PAT 3	120.1	4-6	3-3	2-1-3	4-2
PAT 4	77.1	6-6	3-3	2-2-2	4-2

direction and is valid for studying rest-activity patterns. PIM counts the total amount of motion by the wristwatch accelerometer during the epoch, and was used in this study to look at relationships between skin temperature and activity, because it is a more specific indicator of motion intensity than ZCM [32,33].

2.2. Data analyses

All data analysis was performed using Matlab R2010b (The Mathworks, MA, USA), according to subject group (healthy controls versus patients), IR characterization of skin temperature (warm versus cool) and thermal patch placement (chest versus back; right versus middle versus left). All temperature time series with less than 20 per cent data loss were retained and were subjected to a cubic spline procedure to interpolate lost data points (table 2).

2.2.1. Wavelet analysis to identify temperature rhythm spectra. Time-frequency spectra were generated using

continuous wavelet analysis to obtain increased time resolution at the frequencies of interest in the ultradian and circadian domains, with respective periods of 1-18 h and 18.01-30 h. The gapped wavelet transform (GWT) was used for time-frequency analysis, because it is not limited by a cone of influence that shortens the usable data length at low frequencies, as is the standard continuous wavelet transform [34-36]. A Morlet wavelet with $\omega_0 = 6$ was used as the mother wavelet. Each skin temperature dataset was low-pass filtered with a cut-off frequency of 0.0083 Hz and downsampled to 0.0167 Hz prior to GWT computation. GWT was computed with a frequency range of $0.0081-8.97 \times 10^{-6}$ Hz (period range of 0.03-30 h).

Complex wavelet coefficients were converted to power by taking the square of the absolute value of each coefficient. Wavelet power is related to the temperature variance at each time and frequency location and is therefore presented in units of $^{\circ}\text{C}^2 \text{ period}^{-1}$. The wavelet energy density was computed by taking the area for eight overlapping 6 h ranges staggered by 3 h,

involving periods from 1 to 28 h for the wavelet representation of each signal (range 1: from 1 to 7 h, range 2: from 4 to 10 h, ... and range 8: from 22 to 28 h), and this value was then normalized by dividing by the total area from 1 to 28 h.

2.2.2. Circadian rhythm analysis. We computed the auto-correlation coefficient using a lag-time of 24 h for each signal (i.e. for patch temperature and actigraphy) after normalizing the signal to unit variance and subtracting its mean. This auto-correlation value has previously been termed *r24* for the circadian rhythm analysis of the rest-activity cycle [18,19]. In accordance with the previously reported *r24* analysis, the ZCM actigraphy signal was used for *r24* analysis [18,19]. For all other actigraphy data analysis, PIM was used.

A fast Fourier transform (FFT), with a frequency resolution of 2.03×10^{-6} Hz, was computed for each temperature and rest-activity dataset after subtraction of the mean. If the maximum FFT magnitude existed at a period within a predefined circadian range (18.01–30 h), we considered the dataset had a 'dominant' circadian component.

We fit a sine wave with a period of 24 h to each temperature patch and actigraphy PIM dataset using a nonlinear least-squares approach with the Matlab Curve Fitting Toolbox to determine the 24 h amplitude and phase within 95% confidence limits for each signal. Phase information was used to determine the peak time for each signal.

Correlation coefficients between pairs of skin surface patches were computed for each subject. In addition, correlation coefficients were computed between the ambient temperature and each skin surface patch after skin temperature signals were low-pass filtered to one-half the ambient temperature sampling rate and then downsampled to the sampling rate of ambient temperature.

2.2.3. Statistical analyses. Statistics were computed using the Matlab Statistics Toolbox. Statistical significance was set as $p < 0.05$. Results are displayed as mean \pm s.d. For testing proportion differences between groups, the Pearson χ^2 -test was used. Mean temperatures and 24 h amplitudes as well as normalized wavelets were compared among groups using analysis of variance with a Tukey post hoc test or a paired *t*-test whenever appropriate.

3. RESULTS

Out of 55 skin surface thermal patches placed on subjects, 41 patches (75%) showed less than 20 per cent data loss and were used for subsequent analysis.

3.1. Skin temperature as a biomarker of chronotherapy

Fluctuations in skin temperature occurred throughout monitoring, but beyond the 24 h rhythm there was no trend in skin temperature measured for control subjects. Figure 3*a* shows an example of a control subject's skin temperature signal throughout the monitoring period

where the mean 24 h temperature for all patches on this subject had a slight decrease of $0.14 \pm 0.09^\circ\text{C}$ each day. By contrast, in PAT 3, the skin temperature signal increased by $0.43 \pm 0.04^\circ\text{C}$ each day (figure 3*b*), throughout the administration of chronochemotherapy with chronoFLO4 (figure 3*c*). Actigraphy measurements also revealed rhythm disruption throughout treatment delivery (figure 3*d*) while the rest-activity pattern remained stable in the control subject (figure 3*e*).

To identify ultradian rhythms in skin temperature, we used wavelet analysis to generate a time-frequency spectrum for each temperature signal. This allowed us to visualize the dynamics in skin temperature rhythms. An example of ultradian rhythm for skin temperature is the approximately 2 h rhythm that occurred each night for a control subject's spectra (figure 4*a,c*). This rhythm was less prominent on the chest compared with back locations (figure 4*a,c*).

Variations in ultradian skin temperature rhythms occurred during chronotherapy as shown for PAT 3. Thus, a 24 h rhythm was displayed across the full monitoring period for the example spectrum from a patch with a chest location. Ultradian rhythms with shorter periods also developed on this chest skin patch throughout treatment (figure 4*b*), illustrating changes in skin temperature dynamics at this location throughout the monitoring period. No such temporal modifications were obvious for a patch located on the back of the same subject (figure 4*d*).

All controls and patients had a peak time-averaged wavelet power occur within the circadian region of 18–28 h, except for one control subject, whose peak occurred at approximately 12 h (figure 5*a,b*). A smaller intensity peak at the first harmonic of the circadian rhythm, or a period of approximately 12 h, was recognized for controls, but not the patients.

The majority of normalized wavelet power occurred in the longer period ranges (6, 7 and 8) that overlap the circadian region (figure 6). For controls, ranges 4–8 all hold between 24 and 30 per cent of the total wavelet power followed by 19.6, 14.5 and 9.8 per cent for ranges 3, 2 and 1, respectively. The patient groups had higher normalized power in the circadian ranges 7 and 8 than the controls and lower normalized power in the shorter period ranges 1 through 4, showing an increase in ultradian rhythms for the controls compared with patients (figure 6).

3.2. Characteristics of circadian rhythms in skin temperature and activity

3.2.1. Regularity of circadian rhythm (*r24* analysis). *r24* correlations for ZCM actigraphy signals were on average lower for the patients than for the controls (0.18 ± 0.15 versus 0.45 ± 0.09), with both patients on chronochemotherapy having the lowest scores (0.07 and 0.08), indicating poor reproducibility of 24 h changes from one day to the next. The patient on the standard oral treatment regimen during monitoring had the next lowest *r24* actigraphy (0.20). All controls and the patient without treatment had *r24* actigraphy ranging from 0.3 to 0.6.

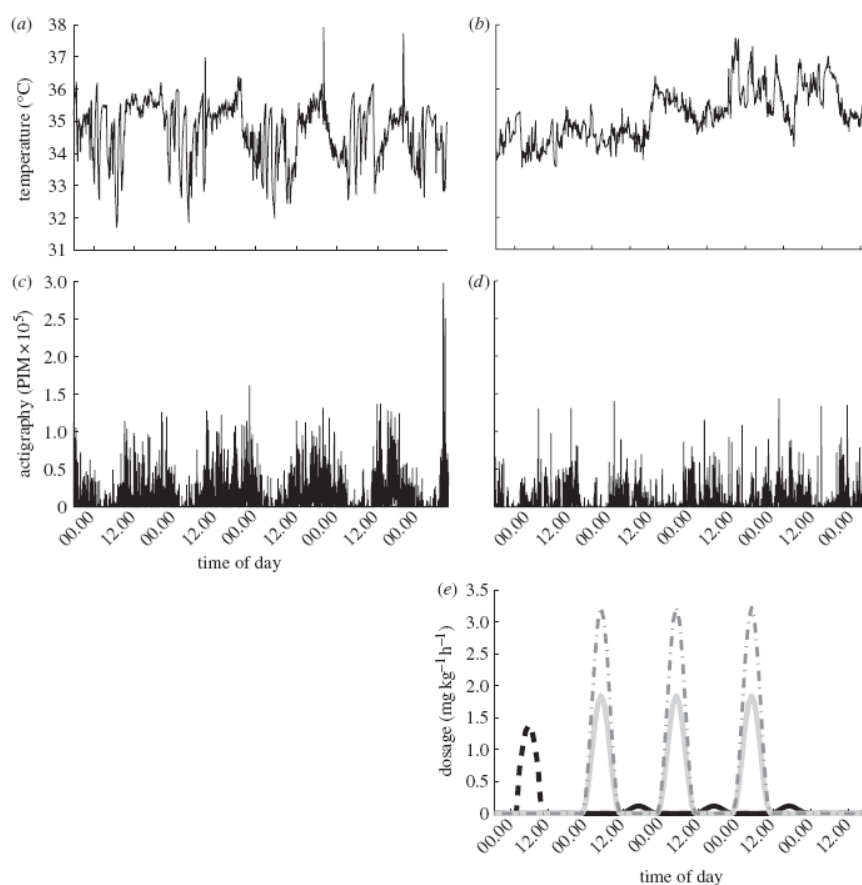


Figure 3. Representative data from (a,c) a control subject and (b,d) a cancer patient receiving (e) intensive chronotherapy with four anti-cancer drugs (ChronoFLO4). Repeatable patterns in the skin temperature from the control acquired from a warm location on the back (a) contrast with the steadily increasing (but with seemingly random changes) skin temperature from a warm location on the chest side of the patient (b) during treatment. Actigraphy patterns (PIM actigraphy data shown) for the control (c) show a consistent rest–activity cycle. Patient activity patterns (d) show sporadic activity, with less rest–awake periodicity compared with the control. The timed ChronoFLO4 infusion schedule for the patient is shown in (e). (e) Dashed black line, CPT; solid black line, OHP; solid grey line, LV; dotted–dashed grey line, 5-FU.

r24 skin temperature values displayed large inter-subject variability. Figure 7 shows r24 skin temperature values for each patch analysed versus the r24 actigraphy in the same subject. The spread in r24 skin temperature was computed for each subject as the difference between the maximum and minimum r24 skin temperature values. The median spread of r24 skin temperature for all subjects was 0.39 (range from 0.18 to 0.57).

3.2.2. Circadian rhythms in skin temperature. The ability to identify a dominant circadian component by using skin surface temperature patches and PIM actigraphy measurements is illustrated in a control for FFT analysis (figure 8a,b) and 24 h curve fitting (figure 8c). Twenty-three out of the 41 skin temperature patches analysed (57%) had their maximum FFT amplitude occur within

the circadian domain. Patches on the left side of the body were more likely to detect the dominant circadian component than patches on the right side ($p < 0.05$), while no difference was found for chest versus back locations or for IR-defined ‘warm’ or ‘cool’ locations (figure 9a). Actigraphy data from all subjects had maximum FFT amplitudes within the circadian domain. This was also the case for ambient temperature for all the subjects, except a patient whose study took place within the hospital setting.

3.2.3. Impact of patch location on circadian temperature amplitude. Twenty-four hour amplitudes from the curve-fitting procedure for each skin temperature signal with a dominant circadian component were averaged together within their respective groups (figure 9b).

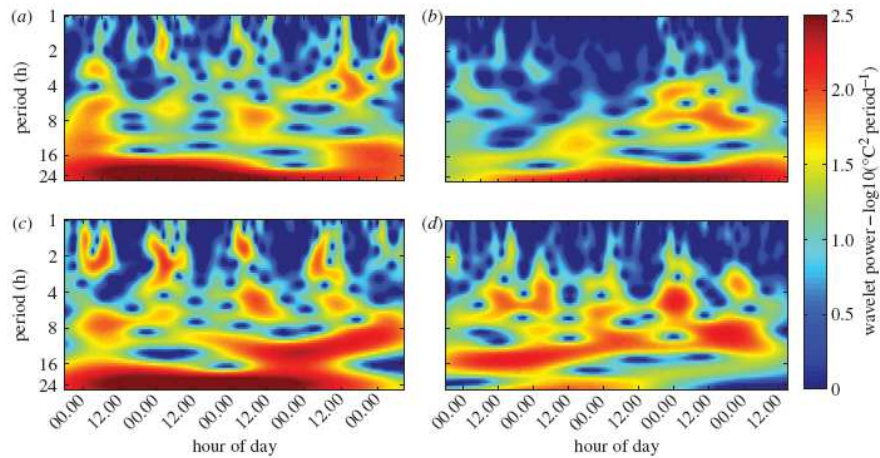


Figure 4. Skin temperature wavelet spectra. Rhythms in skin temperature of less than 24 h are shown in the time–frequency spectra for a thermal patch placed on a chest warm location for (a) a control and (b) a cancer patient. Time–frequency spectra for a back warm location for a control and a cancer patient are shown in (c, d), respectively. Besides the 24 h rhythms present in (a–c), significant power at ultradian rhythms can be seen on all the spectra, with dynamic changes. For instance, the patch shown in (b) for PAT 3 had maximum wavelet power (in periods of less than 16 h) of $20.13^{\circ}\text{C}^2\text{period}^{-1}$, $48.52^{\circ}\text{C}^2\text{period}^{-1}$, $53.06^{\circ}\text{C}^2\text{period}^{-1}$ and $87.54^{\circ}\text{C}^2\text{period}^{-1}$ for the first four 24 h monitoring periods, respectively.

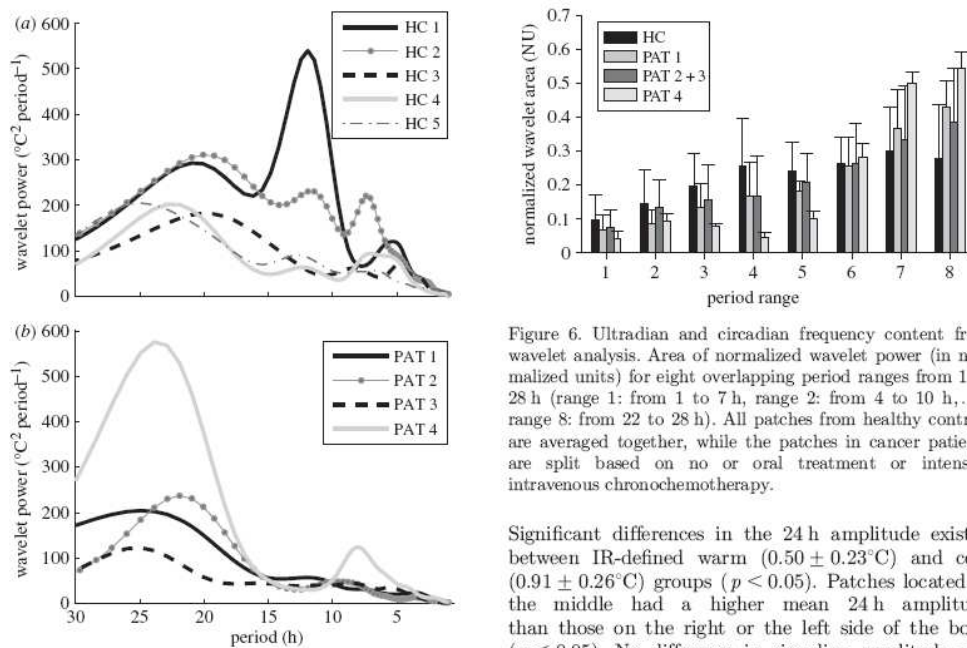


Figure 5. Differences between control and patient time-averaged wavelet spectra. Time-averaged wavelet spectra averaged for all patches for each subject for (a) healthy controls and (b) patients for periods ranging from 1 to 30 h. The only control subject not to have an approximately 24 h dominant period, HC 1, suffered from a chronic infection during monitoring, which may be responsible for the 12 h dominant period seen in (a).

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Figure 6. Ultradian and circadian frequency content from wavelet analysis. Area of normalized wavelet power (in normalized units) for eight overlapping period ranges from 1 to 28 h (range 1: from 1 to 7 h, range 2: from 4 to 10 h, ..., range 8: from 22 to 28 h). All patches from healthy controls are averaged together, while the patches in cancer patients are split based on no or oral treatment or intensive intravenous chronochemotherapy.

Significant differences in the 24 h amplitude existed between IR-defined warm ($0.50 \pm 0.23^{\circ}\text{C}$) and cool ($0.91 \pm 0.26^{\circ}\text{C}$) groups ($p < 0.05$). Patches located in the middle had a higher mean 24 h amplitude than those on the right or the left side of the body ($p < 0.05$). No difference in circadian amplitude was found between the control and patient groups, nor between chest and back locations. Thus, the initial temperature value appeared to be the strongest determinant in the skin temperature circadian amplitude.

3.2.4. Circadian timing in temperature and activity rhythms.

Controls had an average peak time in activity

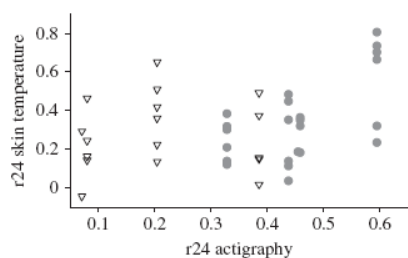


Figure 7. Relations between skin temperature and activity patterns: autocorrelation coefficients. r_{24} skin temperature versus r_{24} actigraphy. r_{24} value from thermal patches for each subject versus the subject's r_{24} value obtained from actigraphy (ZCM). Closed grey circles represent controls, and open triangles represent patients.

at 14.27 h (± 2.5 h). The peak times in activity occurred at 13.48 and 14.45 h in both outpatients, PAT 1 who was not on treatment and PAT 4 who was receiving oral medications. Conversely, a phase advance in activity was apparent for both hospitalized patients on chronoflora, with a maximum occurring at 11.46 h for PAT 2 and PAT 3 (figure 10).

Skin temperature peak times varied throughout the day across subjects. The median daily peak time in the temperature time series of each subject was 21.52 h (± 5.4 h) in controls and 23.39 h (± 2.2 h) in cancer patients. The median difference between the latest and the earliest peak times of the averaged 24 h temperature pattern was similar between control subjects and cancer patients (4.85 and 4.80 h, respectively).

3.3. Thermal characteristics of warm and cool skin areas

Patches with a higher initial mean temperature ($33.31 \pm 0.76^\circ\text{C}$) were designated as warm, and those with a lower mean temperature ($32.18 \pm 0.81^\circ\text{C}$) ($p = 0.001$) were designated as cool. The temperature of the warm and cool patches irrespective of location tended to converge over time, with average temperatures being, respectively, $34.62 \pm 0.85^\circ\text{C}$ and $34.16 \pm 1.06^\circ\text{C}$ ($p = 0.12$). However, qualification of the average temperature for chest or back locations revealed higher values for IR warm patches compared with IR cool patches ($p < 0.001$). The mean temperature did not significantly differ between controls and patients ($p = 0.06$), between chest and back ($p = 0.19$) or among right, middle and left locations ($p = 0.95$).

4. DISCUSSION

Continuously monitoring individual physiological rhythms during chronotherapy is useful not only for determining the circadian phase but also for identifying circadian disruption, either pre-existing or induced by anti-cancer treatments. Both kinds of information regarding the dynamics of the CTS could indeed be critical for the fine tuning of chronotherapeutic delivery

in individual cancer patients, so as to deliver drugs both at their optimal internal timing and proper dose levels. Here, skin surface temperature was monitored for several days in five control subjects and four patients using VitalSense thermal patches. This system was susceptible to data loss during wireless transmission, possibly owing to the location of the monitor outside of the 2 m reception range from activated patches or interference from nearby electronics. We used a cubic spline procedure to fill in lost data points, a procedure that is not expected to impact the current range of interest for periods longer than 1 h. In so doing, 75 per cent of the patches provided relevant time series.

While circadian and ultradian patterns in skin surface temperature were rather stable in individual control subjects, this was not the case for both patients with advanced colorectal cancer receiving a standard intensive four-drug chronotherapy regimen. The modifications in the rhythmic organization of skin surface temperature could result from systemic inflammation, as well as from altered cardiovascular or metabolic processes related to the cytotoxic effects of the anti-cancer drugs.

Ultradian rhythms in skin temperature may be related to cycles in metabolic heat production, changes in skin blood flow, ambient temperature or any combination of these factors. Therefore, monitoring skin temperature rhythms with ultradian components may give us information about the current state of multiple cardiovascular and/or metabolic processes. Wavelet analysis revealed the existence of many ultradian rhythms whose prominence varied along the circadian time scale and along the course of treatment administration in cancer patients. This was supported by the occurrence of significant wavelet power throughout the 1–18 h ranges. Such ultradian components appeared to be more prominent in the controls, rather than in the cancer patients, suggesting that rhythm disruption could further extend to the ultradian domain in cancer patients. The single control not to show a dominant time-averaged wavelet peak in the circadian region, but instead at approximately 12 h, experienced an infectious sinusitis and menstruation phase onset during monitoring. These two processes are known to influence body temperature regulation.

The current study showed that repeatability of circadian rhythms in skin temperature over 24 h (as determined by r_{24} analysis) had a large variability within subjects, dependent upon the location of skin temperature patches. This illustrated the clinically meaningful impact of measurement location for skin temperature-based CTS assessment. This variability appeared independent of how prominent the rest–activity cycle was. Both inpatients monitored during chronomodulated cancer chemotherapy displayed the lowest r_{24} values for the rest–activity rhythm, possibly as a result of the systemic toxicity of anti-cancer drugs. Furthermore, it has been shown that even short-lasting anaesthesia can induce circadian disruption in otherwise healthy subjects [37], pinpointing the sensitivity of the CTS to external pharmacological manipulations. Indeed, the patient not on treatment was undergoing a typical daily routine and had a normal rest–activity

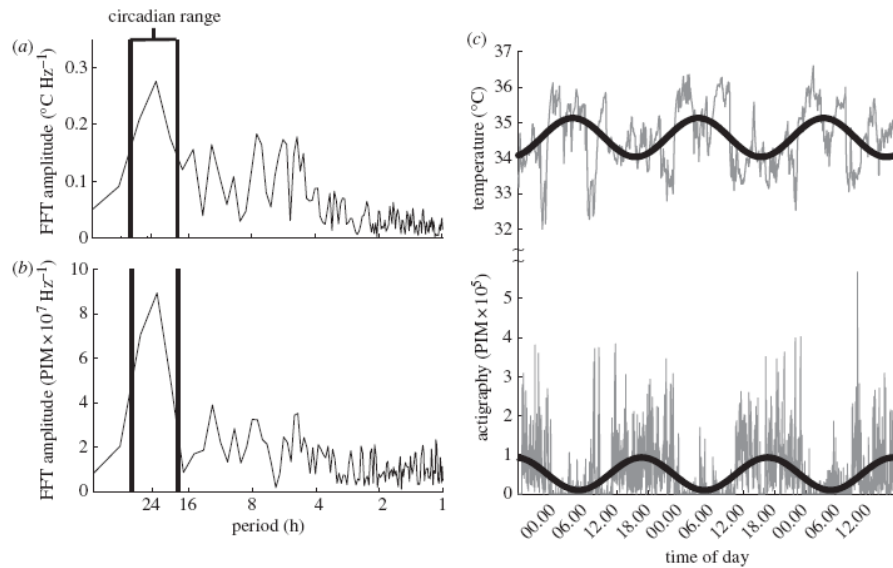


Figure 8. Relations between skin temperature and activity patterns: fast Fourier transforms. Representative FFTs and 24 h curve fitting. Frequency content from periods of 1–40 h for one skin temperature patch (a) and the corresponding actigraphy data (b) for the same subject both show dominant circadian rhythms. Vertical lines in (a,b) represent the pre-set circadian range from periods of 18–30 h. Raw thermal patch dataset (thin grey line) with 24 h sine fit (top thick black line) is shown at the top of (c) for the same signal with frequency spectrum shown in (a). The actigraphy signal (thin grey line) for the FFT in (b) is shown below the skin temperature signal, again with a 24 h sine fit (bottom thick black line).

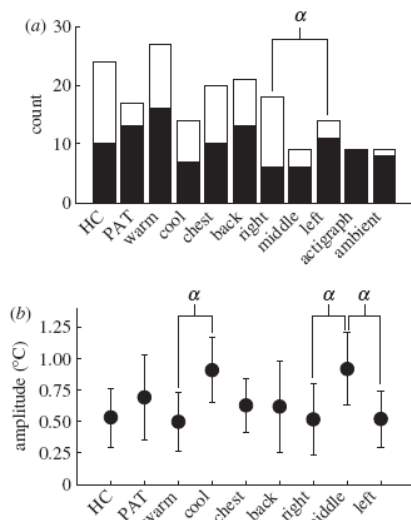


Figure 9. Detection of circadian patterns in skin surface temperature time series. The total number of patches that had a dominant circadian component for each group is shown out of the total number of patches for that group in (a). Mean amplitude for all skin temperature signals with a dominant circadian component, with error bars representing s.d., from the 24 h curve-fitting procedure is shown for each skin temperature group in (b). α denotes significance between marked groups with $p < 0.05$.

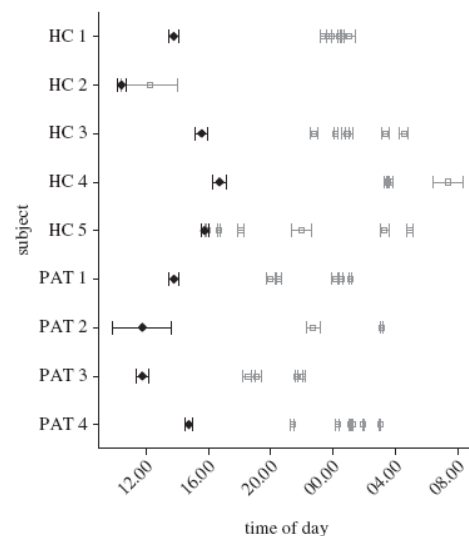


Figure 10. Relations between circadian phases of skin surface temperature and rest-activity. Twenty-four hour peak times for skin temperature and actigraphy data. Time of day of peak 24 h skin temperature (open grey squares) and actigraphy (filled black diamonds) by subject from 24 h curve fitting with 95% confidence limits.

cycle, while the oral medication patient had a slightly disrupted rest–activity cycle.

The high values in activity were similar in controls and in both outpatients receiving no chemotherapy or oral treatment. In contrast, both intensively treated patients on chronoFLO4 displayed an approximately 3 h phase advance of their circadian rest–activity rhythm compared with both outpatients and the control subjects. Such phase advance could relate to the hospital routine, to patient chronotype, an issue not explored in this study, to advanced cancer and/or to chemotherapy toxicity. The circadian phases varied by up to 13 h between patches within a single individual (median of 4.8 h). Thus, measurement of the circadian rhythm at two locations on a single subject may produce two different phases, which may be clinically significant depending on the applications.

Multiple circadian clocks existing in different peripheral tissues with various periods may explain the inter-subject variability that we saw in the circadian rhythms of skin temperature [38,39]. The rest–activity cycle is controlled by the SCN, which is the main circadian oscillator, but peripheral clocks can affect a local tissue region to a greater extent [40,41]. Adipose tissue has been shown to have functional molecular circadian clocks that temporally modulate metabolism, and these could lead to local changes in the circadian rhythm of temperature across the surface of the body [42]. It has also been shown that a change in temperature itself plays an important role in regulating peripheral circadian clocks [27], so it may be that local temperature oscillations are important for fine adjustments in regional peripheral clocks in different tissues.

Previous studies have not taken into account the effect of possible local spatial variations in skin temperature on the circadian component of skin temperature [29,43], but this would only be appropriate if skin temperature were homogeneous across a given surface of the body. In the current study, IR imaging confirmed the heterogeneous pattern of skin temperatures measured at a single circadian time. Furthermore, this is the first time that differences in the circadian rhythm of skin temperature are shown between IR-defined warm and cool locations on the skin of the chest or upper back, with a near doubling of the circadian amplitude for the cool ‘patches’ as compared with the warm ones. Thus, if the goal is to monitor the circadian rhythm and phase over multiple days, then cool locations may be the better choice because the greater circadian temperature variance is a signature of a more prominent circadian rhythm. In addition, multiple locations should be measured to ensure that a location with a dominant circadian rhythm is being captured.

5. CONCLUSIONS

Superficial skin temperature monitoring on the upper torso as a method to determine the circadian phase and to identify disruption of biological rhythms has been investigated. We showed that circadian amplitude and phase in skin temperature vary across measurement

sites for individual subjects, and specifically that locations identified as cool by the use of IR imaging had a larger 24 h amplitude than those identified as warm, pointing to the need for monitoring at multiple sites for accurate determination of the circadian phase. Additionally, in our limited sample size, we found that intensive standardized chronochemotherapy [44] may disrupt both circadian and ultradian rhythms. Recording of skin temperature at different locations might be useful for more precise identification of the individual phase of the CTS of patients and for dynamically tracking biological rhythms to optimize cancer chronotherapeutics. In future studies, temperature monitoring for personalized chronotherapeutics should be performed prior to and during chronotherapy to determine the CTS phase accurately, locate treatment-induced circadian disruption, track specific ultradian rhythms and adjust treatment doses and timing accordingly.

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Le rythme circadien d'activité et repos pendant chimiothérapie pour cancer colorectal métastatique: corrélation avec la toxicité et le devenir du patient

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De rares données cliniques suggèrent l'effet de la chimiothérapie sur le système circadien, qui coordonne les fonctions biologiques sur 24 h. Cette étude précise les relations entre perturbation circadienne sous chimiothérapie, caractéristiques cliniques, principales toxicités et pronostic des patients atteints de cancer colorectal. Le rythme d'activité et repos a été mesuré par actimétrie de poignet dans la semaine suivant l'administration d'une chimiothérapie par 5-fluorouracile, leucovorine et oxaliplatine en première ligne de chimiothérapie pour cancer colorectal métastatique. Il s'agit d'une étude translationnelle annexe à un essai international de phase III comparant une administration chronomodulée à un schéma conventionnel. La fonction circadienne a été estimée par l'index de dichotomie $I < O$, un paramètre robuste et validé. Les données de 77 patients étaient disponibles, avec un enregistrement d'actimétrie réalisé entre la première et la neuvième cure de traitement. Une perturbation du rythme circadien sous chimiothérapie, définie par une valeur de $I < O$ inférieure à 97.5%, a été observée chez 39 patients (51%), et était plus fréquente chez les femmes recevant la chronothérapie (64%). La perturbation du rythme circadien était sélectivement associée à une incidence double de fatigue de grade ≥ 2 et triple de perte de poids $\geq 5\%$. La survenue d'autres toxicités de grade 3-4 ne variait pas selon la robustesse du rythme circadien. La perturbation du rythme circadien pendant la chimiothérapie était un facteur indépendant de mauvais pronostic pour la survie globale, avec un hazard ratio de 1,98 ($p = 0,02$). La chimiothérapie a perturbé le système circadien de près de 50% des patients. La perturbation du rythme circadien sous chimiothérapie était sélectivement associée à une asthénie et une perte de poids, et avec un pronostic plus sombre. La prévention de la perturbation circadienne chimio-induite mérite une évaluation prospective, car elle pourrait conjointement réduire la toxicité des traitements et améliorer la survie des patients cancéreux.

Circadian rest-activity rhythm during chemotherapy for metastatic colorectal cancer: toxicity correlates and clinical outcomes.

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ABSTRACT

Purpose:

Scarce data exist on the effect of chemotherapy on the circadian timing system (CTS), which temporally coordinates biological functions. This study sought to explore the associations between CTS perturbation on chemotherapy and the clinical features, the relevant toxicities and the prognosis of cancer patients.

Methods:

The rest-activity pattern was monitored with wrist actigraphy within one week from the administration of chemotherapy in patients of this companion study of an international Phase III trial comparing chronomodulated vs conventional delivery of 5-fluorouracil, leucovorin and oxaliplatin as first line chemotherapy against metastatic colorectal cancer. Circadian function was estimated with a robust and validated parameter, the dichotomy index I<O

Results:

Data from 77 patients were available, with actigraphy obtained during the first to the ninth course of treatment. Circadian disruption on chemotherapy was observed in 39 patients (51%), and was most frequent in females receiving chronotherapy (64%). Circadian disruption was selectively associated with a double incidence of grade ≥ 2 fatigue and a three-fold increase in body weight loss $\geq 5\%$, and with a similar incidence of grade 3-4 toxicities, as compared to circadian robustness. Circadian disruption during chemotherapy was an independent negative prognostic factor for overall survival, with a Hazard Ratio of 1.98 ($p=0.02$).

Conclusions:

Chemotherapy disrupted the CTS in nearly 50% of the patients. Circadian disruption on chemotherapy was selectively associated with asthenia and body weight loss, and with worse prognosis. The prevention of chemotherapy-induced circadian disruption deserves testing as it could jointly reduce toxicity and improve survival in cancer patients.

KEYWORDS

Circadian Timing System, Chronotherapy, Actigraphy, Cancer, Asthenia, Body Weight, Colorectal, Prognosis.

TEXT

INTRODUCTION

Modern chemotherapy protocols have permitted to prolong the survival of patients with metastatic colorectal cancer [1]. However, these regimens also produce substantial systemic and organ-related toxicities, with a negative impact on patients' wellbeing [2]. In particular, chemotherapy-induced fatigue and anorexia are frequent and bothersome complaints of cancer patients undergoing anticancer treatment, for which no active treatment is available [3-5]. These symptoms cluster frequently in cancer patients, with a possible immune-neuroendocrine mechanism underlying their pathogenesis [6-9]. The scientific evidence supporting this mechanism suggests that a disruption of the circadian timing system could be implicated [3,10-12]. This hierarchically organized system exerts a temporal control over several mind and body functions, at physiological, cellular and molecular levels. The circadian timing system further warrants the administration of anticancer agents at defined times along the 24 hours, so-called chronotherapy [13,14]. The tight temporal coordination of biological functions by the circadian timing system results in slowing down cancer progression both in experimental models and in cancer patients [13-15]. More specifically, median survival was halved in patients with metastatic colorectal cancer with documented baseline circadian disruption, i.e. before any chemotherapy administration, as compared to the patients with a robust baseline circadian rhythm [16,17]. Moreover, several studies have reported a significant association between fatigue, anorexia or weight loss and altered circadian function in patients with various cancers [3,16-22]. However, no systematic assessment has been performed to assess the effect of circadian disruption during chemotherapy on toxicity and disease control. We used data from a prospective companion study to an international randomized Phase III trial to carry out exploratory transversal and longitudinal analyses. The trial involved patients receiving first line chemotherapy for metastatic colorectal cancer. The aim of this study was to identify the toxicity associated with a perturbed circadian system in patients undergoing chemotherapy for metastatic colorectal cancer and the impact of a perturbed circadian system on disease control.

PATIENTS AND METHODS

Study Population

The current study reports the results of an unplanned analysis of prospectively collected data within an international, randomized, controlled open Phase III trial, EORTC 05963 [23]. In this trial, 564 patients with previously untreated metastatic colorectal cancer were enrolled in 36 Institutions, from October 1998 to February 2002. After signing an informed consent, patients were randomized to receive either a 4-day chronomodulated (chronoFLO4) or a 2-day conventional (FOLFOX2) delivery schedule of oxaliplatin, folinic acid and 5-fluorouracil. The trial was approved by the ethics committee in each centre, and was conducted in accordance with the Helsinki declaration. The inclusion and exclusion criteria for this trial, as well as the chemotherapy schedules, have been previously detailed [23]. A subset patients on this study at 9 Institutions (4 Countries) participated in a prospective companion study involving actigraphy monitoring from August 1999 till inclusion was completed [16]. Data from patients participating to this translational research protocol were used for the current study.

Here we report on the associations between the circadian rest-activity rhythm evaluated during and immediately after chemotherapy delivery and the toxicity experienced during the chemotherapy course with actigraphy. Furthermore, we report on the relations between the circadian timing system function during chemotherapy and efficacy outcomes.

Patients included in the current study had their rest-activity pattern monitored with a wrist actigraph within 7 days from the onset of a chemotherapy course, and available toxicity data for this very course. When multiple records were available for the same patient, only the first one was used. A recent publication, further independently confirmed, reported a transient alteration in circadian rest/activity rhythm during the first week of chemotherapy, with a subsequent recovery (Ortiz-Tudela E. and collaborators, manuscript in preparation) [24]. Based on these reports, we used data that was obtained during the first week following chemotherapy administration.

Actigraphy Recording and Circadian Rest/Activity Rhythm Assessment

Individual locomotor activity was monitored noninvasively using a Mini-Motionlogger actigraph (Ambulatory Monitoring Inc., USA): a watch-sized piezoelectric linear accelerometer with a memory chip for data storage that continuously records the number of nondominant-wrist accelerations per minute during at least 72 hours [16,17,25,26]. Data from actigraphy time series were analyzed using a dedicated program (Action 4, version 1.10; Ambulatory Monitoring Inc., USA) to provide a robust, well-characterized and clinically meaningful parameter, the dichotomy index (I<O), to estimate individual circadian rest/activity pattern, [16-18]. The dichotomy index provides an integration of the circadian regulation of sleep and activity, and represents the percentage of minutes during the rest span when activity is lower than the median activity during wakefulness [27]: in case of a robust and marked circadian rest-activity pattern I<O reaches 100%. The lower the I<O value, the more severe the disruption of the circadian rest-activity rhythm. Indeed, baseline I<O values that were 97.5% or less before chemotherapy significantly predicted for lower tumor response rate, shorter progression-free survival and worse overall survival in a pooled analysis of 436 patients with metastatic colorectal cancer (Lévi F. and collaborators, personal communication). Here, we considered that patients with I<O lower than or equal to 97.5% had an altered circadian rest-activity rhythm, that reflected a clinically-relevant disruption of the circadian timing system.

Toxicity Assessment

Clinical and hematological toxicities were assessed at least fortnightly, and graded according to the National Cancer Institute Common Toxicity Criteria, version 2.0. Patient's weight was measured before each course was administered. The toxicity data used here were limited to the worst grade of each toxicity experienced by the patient during the interval between the beginning of the course preceding and that following actigraphy.

Hematological (anemia, thrombocytopenia, leucopenia and neutropenia) and clinical toxicities (diarrhea, mucositis, hand-foot syndrome, peripheral sensory neuropathy, nausea and vomiting, fatigue and body weight loss) were assessed. No data were available for anorexia. Based upon previous reports showing a significant association between fatigue and weight loss with altered locomotor activity rhythm before or off chemotherapy [3,16-19], we considered these two events separately from the other toxicities, for which no association with circadian dysfunction has been reported to date. Clinical relevance was attributed to grade 2 or 3 fatigue [28] or grade 1 body weight loss,

corresponding to 5% or more within 2 weeks [29]. The other toxicities were considered as clinically relevant if they were of grade 3 or more.

Clinical outcomes

Overall incidence per patient of severe or life-threatening toxicity was calculated throughout the treatment span, according to the National Cancer Institute Common Toxicity Criteria, version 2.0. The best objective tumor response obtained throughout the treatment was evaluated and rated according to the WHO criteria. Overall and progression-free survival durations were computed from the beginning of actigraphy recording. Patients lost to follow-up were censored at the last contact date.

Statistical Analyses

The main endpoint of this study was to describe the clinical features and the toxicities associated with chemotherapy-induced circadian disruption. Moreover, the study aimed at investigating the relationship between chemotherapy-induced circadian disruption and patient outcomes. Exploratory analyses were performed with a two-sided Fisher's exact test for categorical variables and with a Mann-Whitney U test for quantitative parameters, in order to compare the various clinical features or toxic events between the subgroup of patients with circadian disruption and that with robust circadian patterns, using the cut-off value of 97.5% for I<O. Multivariate binary logistic regression was used to explore the clinical features and the toxicity events associated with circadian disruption during chemotherapy. The logrank was used to compare the survival estimates obtained with a Kaplan-Meier method, according to circadian disruption versus robustness. Cox multivariate hazard modeling was performed adding gender, treatment schedule, number of metastatic sites, rank of chemotherapy cycle with actigraphy and Performance Status at day1 of the course as fixed covariates in order to explore the independent prognostic value of circadian disruption during chemotherapy. The assumption of a normal distribution of I<O was tested using the Kolmogorov-Smirnov test. Missing data for any parameter were not imputed, and percentages reported here refer to available data for each variable. The threshold for statistical significance was set at 5%. Analyses were performed using PASW Statistics 18 software (SPSS Inc., USA).

RESULTS

Clinical and Demographical Characteristics

Seventy-seven patients fulfilled the inclusion criteria, with actigraphy being recorded during or within the week following the administration of a chemotherapy course. This represented 13.7% of the whole population of the patients registered in the Phase III trial, and 40.3% of those enrolled at the institutions participating in this companion study. Table 1 summarizes the main clinical and demographical characteristics and the overall outcomes of the current study patients, which were similar to those of the whole trial population [23] (Table 1).

I<O distribution

The distribution of I<O was not normal ($p<0.0001$). The respective median and mean values of I<O reached 97.5% (interquartile range, 5.3%) and 95.1% (standard deviation, 7.8%) (Figure 2A). The median I<O value corresponded exactly to the cut-off value previously selected for defining circadian disruption. Thus, the 39 patients with altered circadian rest-activity rhythm (i.e., I<O lower than or equal to 97.5%) represented 50.6% of the current study population (Table 1; Figure 1A). Patients with circadian disruption displayed a median I<O of 93.8% (range: 42.3% to 97.5%), whilst the median I<O value of the patients with persistent circadian rest/activity rhythm was 98.9% (range: 97.6% to 100%). Overall, the distribution pattern of I<O on treatment strongly resembled that reported at baseline in two independent cohorts respectively involving 130 and 170 patients with metastatic colorectal cancer patients [16-18] (Figure 1B).

Timing of Actigraphy Monitoring

A median of five chemotherapy courses (range, 1 to 9) was given before the current "on treatment" wrist-actigraphy monitoring (Figure 2A). Recording took place during chemotherapy delivery for 80.5% of the patients. The median interval between the onset of actigraphy monitoring and that of chemotherapy was one day (Figure 2B).

Toxicity and missing data

Overall, 12 patients (16.0 %) experienced grade ≥ 2 fatigue, and 4 patients (5.6 %) lost 5% or more of their body weight, after the chemotherapy course of interest. Grade ≥ 3 clinical or hematological toxicities occurred in 10 patients (13.0 %), with each kind of severe toxicity being infrequent (Table 2). Body weight loss $\geq 5\%$ was associated with the occurrence of grade ≥ 2 asthenia or with grade ≥ 3 toxicities in 2 patients each. In contrast, clinically relevant fatigue jointly occurred with other toxicities in 4 patients. Thirty-four patients (44.2%) displayed grade 2-4 clinical or hematological toxicities besides asthenia or body weight loss. Performance status worsened in 6 patients (8.2 %), and improved in 5 patients (6.8 %) after chemotherapy (Table 1).

The overall rate of missing information on toxicity, performance status or body weight was 27 out of 1155 variables (2.3%), with at least one missing parameter value in 19 patients (24.7%).

Circadian Disruption and Clinical Features

The baseline clinical characteristics at study entry were similar regardless of the subsequent occurrence of circadian disruption on chemotherapy (Table 1). No difference in any feature related to the chemotherapy course of interest was observed between patients with circadian disruption and those with a robust circadian rhythm (Table 1). The duration of the course of chemotherapy of interest, and the related rates of cycle delay or dose modifications, were similar in the subgroups of patients with circadian disruption or with marked circadian function (Table 1). Exploratory statistical comparisons between patients with robust or altered circadian rest-activity rhythm on chemotherapy showed no significant difference according to age ($p=0.48$), PS ($p=0.60$), BMI ($p=0.65$), number of metastatic sites ($p=0.48$), site of primary tumor ($p=0.49$), actual administered dose of oxaliplatin ($p=0.99$) or 5-fluorouracil ($p=0.93$), PS on first day of the treatment course of interest ($p=0.22$), course rank ($p=0.46$), duration of the chemotherapy course ($p=0.78$), and timing of actigraphy ($p=0.45$). The incidence of circadian disruption during chemotherapy did not significantly differ according to gender and randomized treatment ($p=0.71$) (Figure 3A); thus, the proportion of patients altered circadian rest/activity rhythm was 48.0% in males and 55.6% in females ($p=0.64$), and 48.7% and 52.6% in patients on FOLFOX2 and chronoFLO4 ($p=0.82$). However, the highest incidence of circadian disruption was observed in females on chronoFLO4 (64.3%) as compared to the other three subgroups ($\leq 50.0\%$) (Figure 3A).

Circadian Disruption and Toxicity

The incidence of circadian disruption during chemotherapy was comparable among patients whose PS deteriorated (50.0%), remained unchanged (46.8%) or improved (60.0%) following the administration of the chemotherapy course of interest ($p=0.85$). The low incidence of clinically-relevant fatigue and body weight loss precluded any statistical validation of differences according to I<O values ($p=0.35$). However, chemotherapy-induced fatigue of grade 2 or higher occurred twice as often in patients with circadian disruption ($n=8$; 21.1%) as compared to those with a robust circadian rhythm ($n=4$; 10.8%) (Figure 3B). Similarly, body weight loss $\geq 5\%$ occurred three times as frequently in patients with altered rest-activity circadian rhythm ($n=3$; 8.6%) as compared to those with a robust rhythm ($n=1$; 2.7%) (Figure 3B). The incidence of other grade ≥ 3 clinical or hematological toxicities during the chemotherapy course of interest was similar in patients with low I<O ($n=5$; 12.8%) or high I<O ($n=5$; 13.2%) ($p=0.96$) (Figure 3B). Overall, absent or mild toxicities were more frequent in patients with a robust circadian rhythm despite chemotherapy as compared to patients with a disrupted circadian rest/activity rhythm (65.8% vs 46.2%; $p=0.11$).

Factors predicting the occurrence of circadian disruption during chemotherapy

Further exploratory analysis was performed in order to determine the clinical characteristics or the toxicities potentially associated with the occurrence of circadian disruption during chemotherapy. The univariate and multivariate binary logistic regressions failed to identify any parameter significantly predicting for the occurrence of altered circadian rest-activity rhythm on treatment ($p \geq 0.13$; not shown).

Baseline actigraphy was available before chemotherapy start for 44 of the 77 patients. Half of them ($n=22$) displayed a disrupted circadian rest-activity rhythm, with I<O lower than or equal to 97.5%. Baseline circadian disruption did not predict for subsequent circadian disruption during chemotherapy ($p=0.23$; not shown). No significant correlation was found between baseline and 'on treatment' I<O values ($r=0.15$; $p=0.35$).

Circadian disruption during chemotherapy and clinical outcomes

No statistically significant difference was found for objective response rate ($p=0.82$), progression-free survival ($p=0.79$) and overall grade 3-4 toxicity rate ($p=0.80$) according to I<O (Table 1). Conversely, overall survival was significantly longer in patients with a robust circadian rhythm as compared to those with circadian disruption ($p=0.013$) (Figure 4; Table 1). The negative prognostic value of circadian disruption during chemotherapy remained statistically significant after adjustment for gender, treatment schedule, number of metastatic sites, rank of chemotherapy course of interest and PS on day1 ($p=0.02$). Thus, patients with an altered rest-activity circadian rhythm during chemotherapy displayed an independent, almost doubled, risk of an earlier death following actigraphy monitoring as compared to patients with a robust circadian rhythm (Hazard Ratio: 1.98; 95% CI: 1.18 - 3.31).

DISCUSSION

In this international study we explored the associations between circadian disruption during chemotherapy and treatment-induced adverse events and clinical outcomes. The function of the circadian timing system was estimated using the non-invasive recording of locomotor activity with a wrist-actigraph, and the computation of a robust and validated circadian parameter, the dichotomy index (I<O) [16-18,27]. Data from a pooled analysis of 436 patients with metastatic colorectal cancer allowed the identification of a clinically relevant cut-off value for I<O for assuming circadian disruption (Lévi F. and collaborators, personal communication).

A transversal design was used for the first time, to describe and compare the incidence of chemotherapy-induced toxicities between patients with robust or altered circadian timing system. Indeed, a repeated measures approach has been used by other researchers to explore the dynamics of circadian or sleep functions during treatment, and their correlations with the temporal evolution of a subjectively-rated symptoms, mainly fatigue [19,21,24,30-32]. These studies have consistently described an inverse correlation between the patterns of fatigue and those of physical activity or sleep efficiency over time [19,21,24,30-32]. Instead, our study has looked at the incidence of clinically relevant toxicities occurring during the chemotherapy course when circadian rhythm evaluation was performed. This approach was motivated by the paucity of data reporting the clinical impact of circadian disruption during chemotherapy. Indeed, previous studies in experimental models and in cancer patients have shown that chemotherapy can disrupt host circadian rhythms [14,20,21,24,33]. This novel and hitherto overlooked toxicity could potentially hinder the anticancer activity and the safety of cancer chronotherapy whose foundations assume consistent and predictable circadian rhythms in those physiological and molecular parameters which mediate pharmacological effects and cancer proliferation [13,14,34]. The subgroup which exhibited the highest rate of chemotherapy-induced circadian disruption in the current study (Figure 3A) and the worst clinical outcomes in the phase-III trial [23] were females treated with chronofol. This finding supports the hypothesis that gender-related circadian differences in humans [35-37] lead to gender-dependent survival and tolerance disparities on chronotherapy [23,38], thus implying that the best chronotherapy schedule differs between men and women.

Both, the limited sample size of our study and the low incidence of adverse events preclude any statistical validation of differences in adverse events according to I<O. However, fatigue and weight loss occurred selectively and largely more often in patients with circadian disruption as compared to those with a robust circadian timing system (Figure 3B). These findings concur with other studies that have reported an association between circadian disruption and fatigue, anorexia and weight loss in cancer patients and in healthy subjects intolerant to shift work or jet lag [3,16-19,39,40]. These symptoms frequently cluster as a consequence of the burden of cancer and in association with toxic chemotherapy [3,41-44]. This suggests that shared pathophysiological mechanisms could underlie their development. The circadian timing system, which temporally coordinates important physiological functions, including physical performance and appetite [3,13,14,34], could, when disrupted, be one of the main determinants in the pathogenesis of this symptom cluster, and a potential target for its treatment [3].

The clinical relevance of chemotherapy-induced circadian disruption is not simply limited to the association with these systemic toxicities. Indeed, the overall survival of patients with altered rest-activity circadian rhythm during chemotherapy was shown here, for the first time, to be significantly shorter than that of patients with a robust circadian rhythm (Figure 4), independently from other prognostic factors or the timing of wrist-actigraphy assessment. This result extends those already documenting the independent prognostic value of baseline I<O for the overall survival of patients with metastatic colorectal cancer [16,17]. This finding supports the hypothesis that undue chemotherapy-induced circadian disruption could engender poorer cancer control by the host, thus shortening overall survival [14].

Conversely, an improvement in circadian function during chemotherapy could reflect improved control of tumor growth by the host, with an associated better prognosis, as shown experimentally [15].

Another limitation of the current study is related to the heterogeneity in the timing of actigraphy monitoring in relation to treatment, in terms of course rank and toxicity assessment. However, no difference in the incidence of circadian disruption was found with regard to treatment timing (Table 1), supporting the merging of all available data. Moreover, this transversal approach gives a unique global assessment along treatment span on the clinical and toxic correlates of circadian disruption during chemotherapy. However, whilst actigraphy monitoring was performed for 72 hours during the initial week of the chemotherapy course, the precise timing of occurrence of the assessed toxicities was unknown and spanned along the whole duration of the treatment course and subsequent treatment-free interval (Table 1). The possible timing lag between the occurrence of the toxic event and that of chemotherapy-induced circadian disruption could not be accounted for in the current study and could have potentially underestimated the delved associations between circadian disruption and toxicity. The current results support the future development of prospective and large studies using a continuous and repeated measures approach, as currently conducted in a companion translational study to the European phase II trial OPTILIV07 (ClinicalTrials.gov Identifier: NCT00852228).

In conclusion, we report here a higher incidence of clinically relevant fatigue and weight loss, and a shortened survival, in patients with altered circadian function during chemotherapy administration. The study provides quantitative estimates that will enable and warrant the tailored design of a sufficiently powered prospective study, in order to confirm the relevance of circadian disruption for selected symptoms and survival in cancer patients receiving chemotherapy and to prevent its occurrence. The shielding the circadian timing system from chemotherapy-induced alterations, through chronotherapy optimization and/or specific pharmacological targeting, could become a novel therapeutic objective for achieving simultaneously reduction of systemic symptoms and improvement of survival in cancer patients on chemotherapy [14,16,45].

TABLES

Table 1.

Clinical and demographical characteristics of the 77 patients of the current study, according to the presence or absence of circadian disruption during chemotherapy.

Feature		Circadian disruption		Total study population (n=77)
		No (n=38)	Yes (n=39)	
Treatment arm (%)	FOLFOX2	52.6	48.7	50.6
	chronoFLO4	47.4	51.3	49.4
Gender (%)	Females	31.6	38.5	35.1
	Males	68.4	61.5	64.9
Age (years)	Median (Range)	60.5 (33.2-75.5)	64.3 (27.4-75.0)	62.3 (27.4-75.5)
	1 st - 3 rd quartiles	54.1-66.3	52.2-69.3	52.9-68.4
Site of primary tumor (%)	Colon	76.3	78.9	77.6
	Rectum	23.7	21.1	22.4
Number of metastatic sites (%)	1	31.6	42.1	36.8
	2	52.6	34.2	43.4
	≥ 3	15.8	23.7	19.7
PS (WHO) at study entry (%)	0	76.3	66.7	72.4
	1	18.4	28.2	22.4
	2	5.3	5.1	5.3
BMI (Kg/m ²) at study entry (%)	Normal (18.5-24.9)	42.1	50.0	46.1
	Abnormal	57.9	50.0	53.9
Number of chemotherapy cycle of actigraphy recording	Median (range)	4 (1-9)	4 (1-9)	4 (1-9)
	1 st - 3 rd quartiles	2-5	2-5	2-5
Interval between actigraphy recording and	Median (range)	2 (1-6)	1 (0-7)	1 (-1-7)

chemotherapy cycle start (days)	1 st - 3 rd quartiles	0-4	0-5	0-4
PS at the cycle of actigraphy monitoring (%)	0	63.2	56.4	59.7
	1	36.8	35.9	36.4
	2	0	7.7	3.9
PS change following actigraphy recording (%)	Worsening	7.9	8.6	6.8
	No change	86.8	82.9	84.9
	Improvement	5.3	8.6	8.2
Administered dose of Oxaliplatin (mg/sqm) at the cycle of actigraphy recording	Median (range)	100 (0-107)	100 (80-107)	100 (0-107)
	1 st - 3 rd quartiles	99-100	100-100	99-100
Administered dose of 5-Fluorouracil (g/sqm) at the cycle of actigraphy recording	Median (range)	3.40 (2.20-3.71)	3.40 (1.83-3.66)	3.40 (1.83-3.71)
	1 st - 3 rd quartiles	3.00-3.60	3.06-3.60	3.00-3.60
Course duration (days)	Median (range)	14 (12-49)	14 (13-29)	14 (12-49)
	1 st - 3 rd quartiles	14-19	14-21	14-21
Oxaliplatin dose reduction following the cycle of actigraphy recording (%)	Yes	13.2	13.5	13.3
5-Fluorouracil dose modification following the cycle of actigraphy recording (%)	Reduction	15.8	10.8	13.3
	Escalation	21.1	21.6	21.3
Objective Response (%)	Yes	52.6	47.4	50.0
Progression Free Survival (months)	Median (95% CL)	8.2 (6.2-10.3)	7.3 (4.2-10.3)	7.9 (6.9-8.8)
Overall Survival (months)	Median (95% CL)	22.3 (19.6-25.1)	14.7 (11.8-17.6)	20.2 (14.4-26.0)
Overall G≥3 Toxicity (%)	Yes	73.7	71.1	72.4

Table 2.

Incidence of severe toxicity in the chemotherapy course of actigraphy assessment according to the presence or absence of circadian disruption during chemotherapy.

G ≥ 3 Toxicity	Circadian rest/activity rhythm		Total (n=77)
	Marked (n=38)	Disrupted (n=39)	
	N (%)		
Anemia	0	0	0
Leucopenia	0	1 (2.6)	1 (1.3)
Neutropenia	0	1 (2.9)	1 (1.5)
Thrombopenia	0	0	0
Diarrhoea	4 (10.8)	1 (2.6)	5 (6.6)
Stomatitis	1 (2.7)	0	1 (1.3)
Hand-Foot Syndrome	0	0	0
Nausea-Vomiting	0	2 (5.1)	2 (2.6)
Sensory Neuropathy	0	0	0
TOTAL	5 (13.2)	5 (12.8)	10 (13.0)

FIGURE LEGENDS

Figure 1.

Timing of actigraphy monitoring in relation to chemotherapy. Panel A: cycle number following which locomotor activity was recorded. Panel B: interval in days between the beginning of the administration of the chemotherapy course and the beginning of wrist-actigraphy monitoring.

Figure 2.

Panel A: distribution of the parameter I<O in the current study population. I<O values ≤ 97.5% define circadian disruption and are in the grey area. Each dot represents a patient, and the horizontal line shows the median value (97.5%). Panel B: boxplots of the distribution patterns of I<O in the current study population (left) and, as a comparison, in two separate populations of patients with metastatic colorectal cancer whose locomotor activity was recorded before starting treatment [16,17]. The bars represent the median with the 1st and 3rd quartiles, and the whiskers show the range.

Figure 3.

Panel A: proportions of patients with disrupted circadian rest/activity rhythm in the patient subgroups defined by the randomized treatment schedule, gender, and gender x treatment. Panel B: incidence of clinically relevant toxicities in the subgroups of patients with marked (left, white bars) or disrupted (right, grey bars) circadian rest/activity rhythm.

Figure 4.

Kaplan-Meier curves depicting overall survival, computed from the beginning of actigraphy recording, according to the persistence of marked circadian function (black dashed line) or the occurrence of circadian disruption (solid gray line). P value derived from a logrank test. Hazard ratio derived from a Cox proportional hazard model.

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CONFLICT OF INTEREST DISCLOSURE STATEMENT

All authors declare to have no conflicts of interest that could have potentially biased the conception or design of the study, or the acquisition, analysis or interpretation of data, or the report of the results.

FIGURE 1.

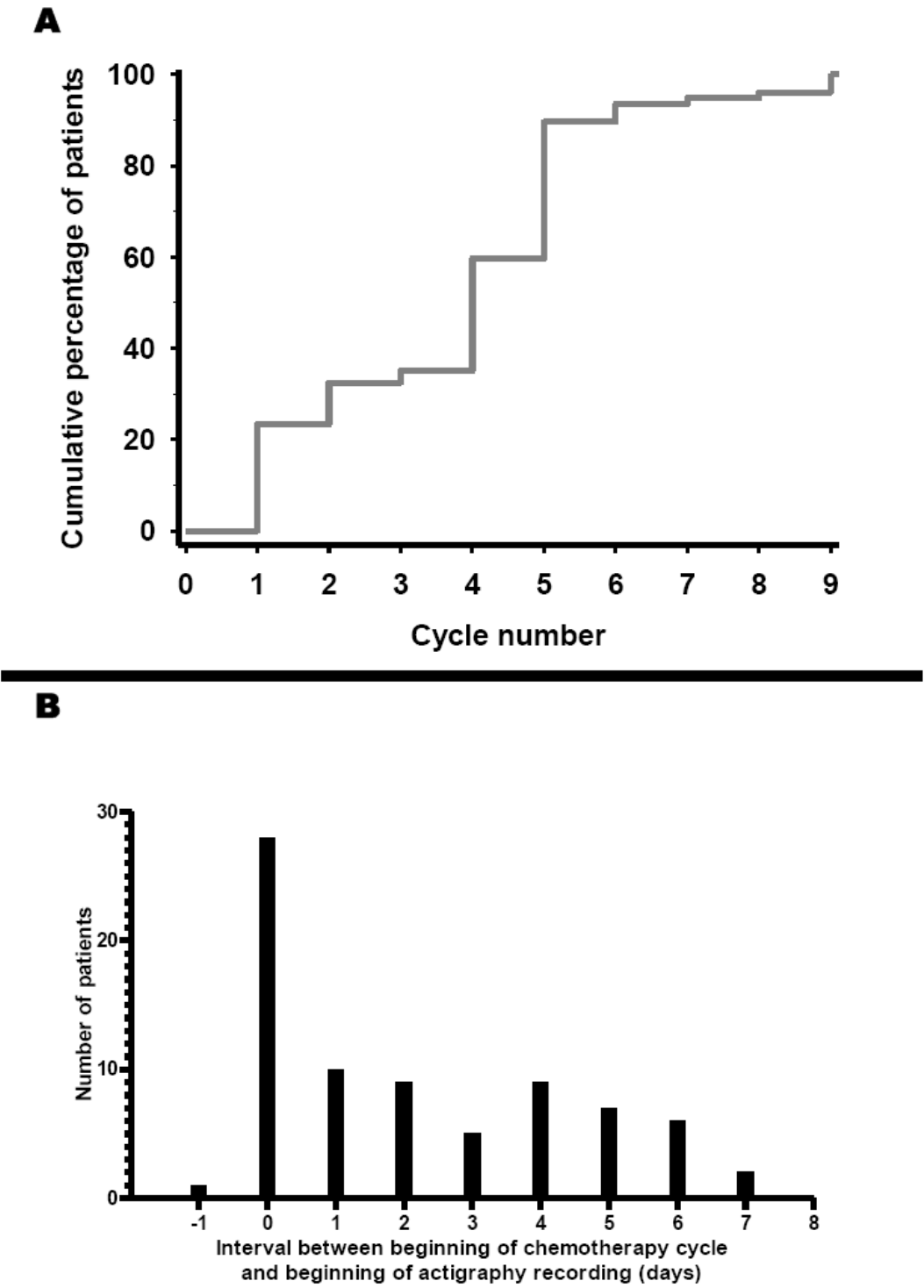


FIGURE 2.

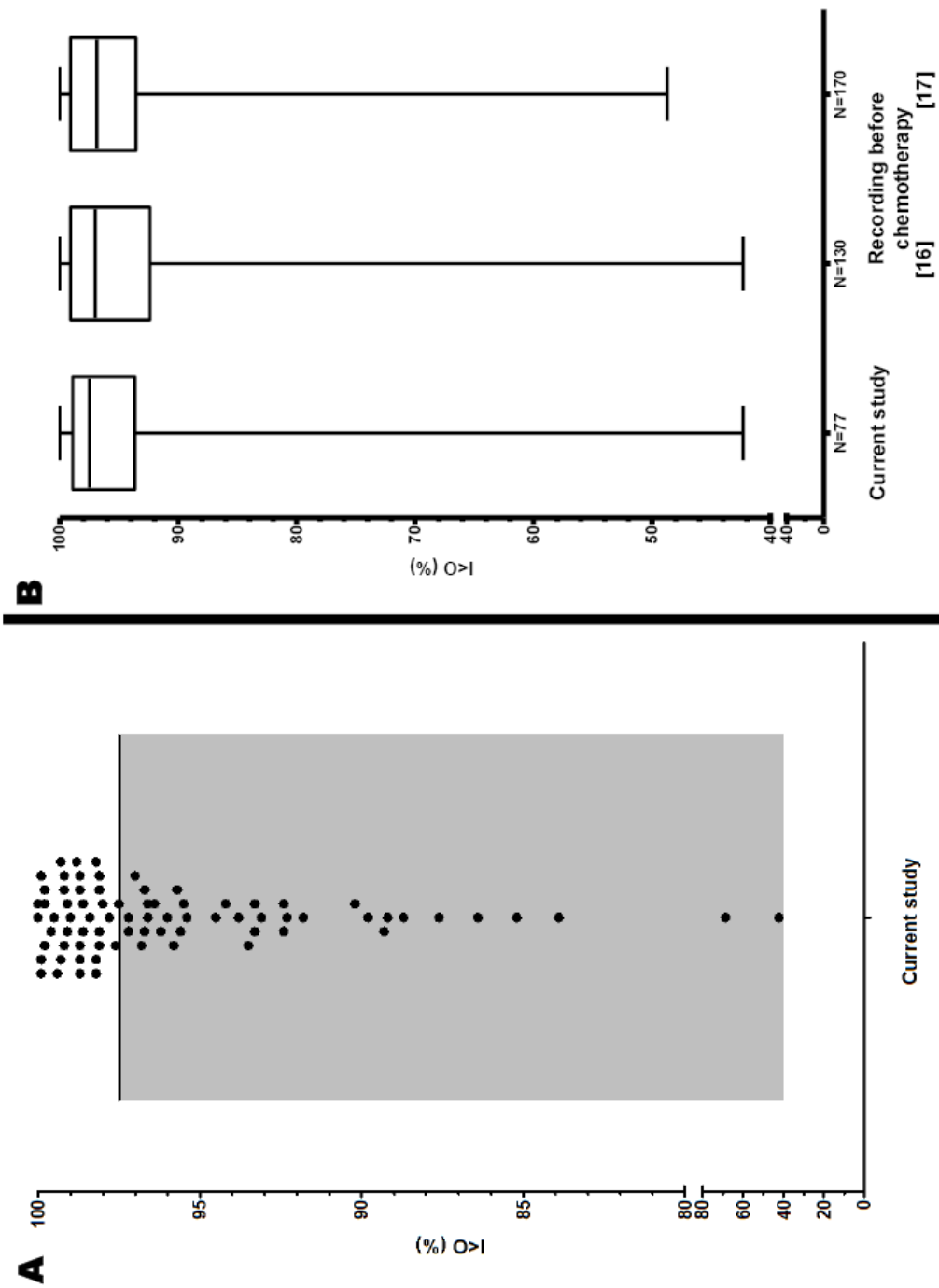


FIGURE 3.

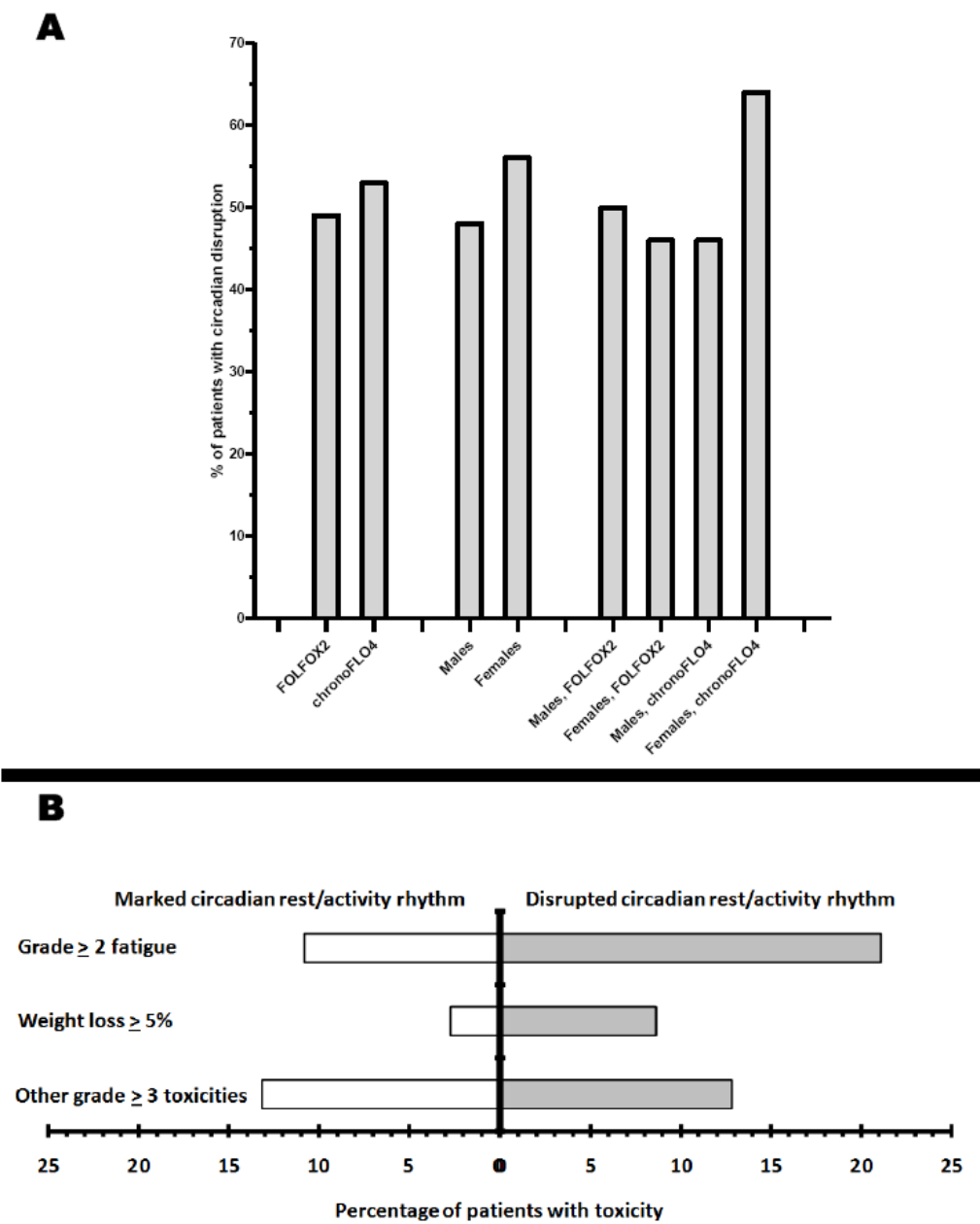
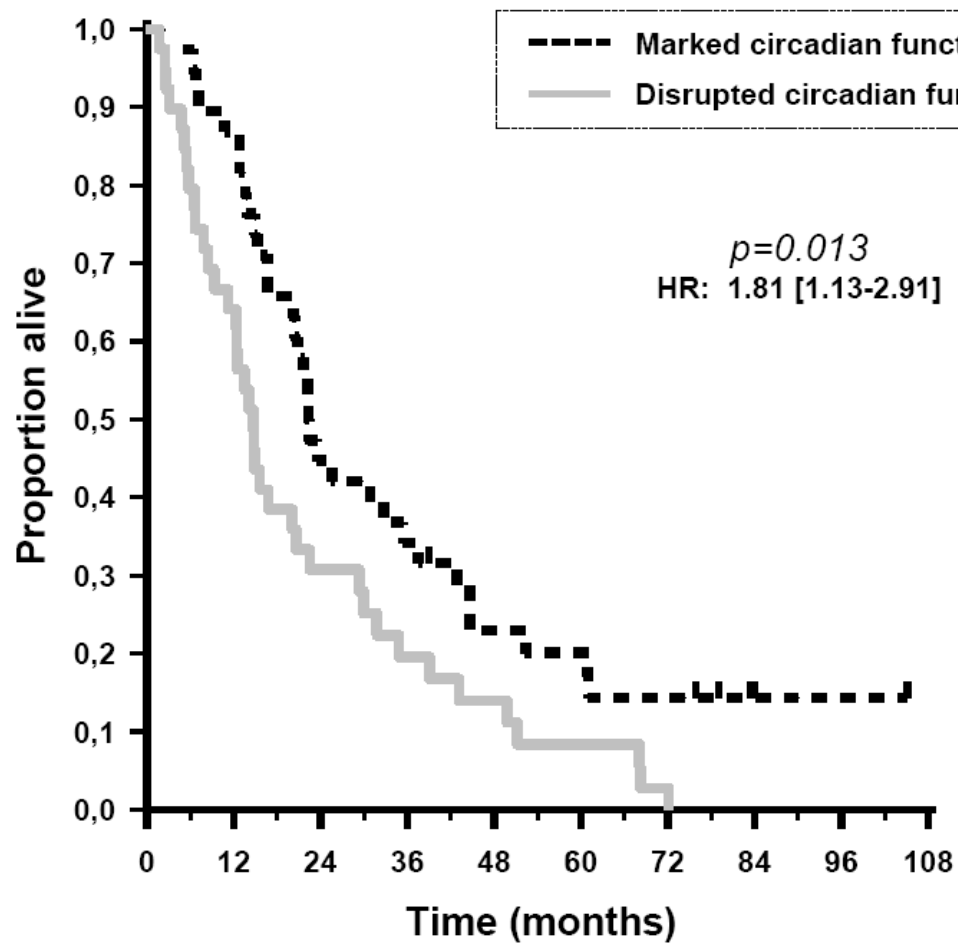


FIGURE 4.



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V.4. Relation entre activité et tolérance de la chronothérapie: rôle du sexe

V.4.1. Article # 9, soumis pour publication

Modification des effets de la chronothérapie sur la survie selon le sexe, chez les patients atteints de cancer colorectal métastatique: une méta-analyse.

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Contexte: Les horloges circadiennes moléculaires modifient la toxicité et l'efficacité de la chimiothérapie anticancéreuse, avec une possible différence selon le sexe.

Objectif: Explorer si le sexe permet de définir le schéma optimal d'administration de l'association 5-Fluorouracile, Leucovorine et Oxaliplatine, en première ligne de chimiothérapie du cancer colorectal métastatique.

Méthodes: Une méta-analyse a porté sur les données de trois essais internationaux randomisés de Phase III qui comparaient les perfusions de ces 3 médicaments en administration chronomodulée (chronoFLO) ou conventionnelle (CONV). Les données de 345 femmes et 497 hommes ont été mises à jour à 9 ans. Le critère principal était la survie globale. Les effets du schéma thérapeutique ont été étudiés en fonction du sexe à l'aide de modèles de Cox et d'analyses multivariées.

Résultats: Il n'existait aucune différence de survie globale liée au schéma d'administration ou au sexe dans la population globale. Cependant la survie globale était prolongée chez les hommes sous chronoFLO par rapport à ceux traités par CONV ($p = 0,009$), avec des valeurs médianes respectives de 20.8 mois [Limites de Confiance à 95 %, 18,7 à 22,9] et 17,5 mois [16,1 à 18,8]. Inversement, chez les femmes, la survie médiane était de 16,6 mois [13,9 à 19,3] sous chronoFLO et de 18,4 mois [6,6 à 20,2] sous CONV ($p = 0.012$). L'interaction entre sexe et schéma d'administration était un facteur prédictif robuste du schéma thérapeutique le plus efficace, avec des rapports de risque de 1.4 [1.05-1.86] pour la survie sans

progression ($p = 0.021$) et de 1.59 [1.30-1.75] pour la survie globale ($p = 0.002$) selon les analyses multivariées.

Conclusions: Cette méta-analyse montre que les hommes vivent significativement plus longtemps avec une chimiothérapie administrée de façon chronomodulée plutôt que selon une modalité conventionnelle. Il est remarquable que les horaires d'administration de chronoFLO chez les patients étaient sélectionnés à partir de résultats d'expérimentations circadiennes chez les souris mâles et d'études translationnelles chez les hommes. Des recherches spécifiques concernent maintenant le problème de l'horaire circadien optimal de la chronothérapie chez les femmes. Le schéma chronoFLO testé ici devrait être recommandé pour le traitement de première ligne des hommes atteints de cancer colorectal métastatique.

Sex Moderates Circadian Chemotherapy Effects on Survival of Patients with Metastatic Colorectal Cancer: A meta-analysis.

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Abstract

Context: There is evidence that molecular circadian clocks modify toxicity and efficacy of cancer chemotherapy, with a possible moderation according to sex differences.

Purpose: To investigate whether sex determines the optimal delivery schedule of 5-Fluorouracil, Leucovorin and Oxaliplatin as first line treatment for metastatic colorectal cancer.

Methods: A meta-analysis was carried out using data from three international randomized

Phase III trials comparing infusional 5-Fluorouracil, Leucovorin and oxaliplatin administered in chronomodulated (chronoFLO) or conventional (CONV) fashion. The data from 345 females and 497 males were updated at 9 years. The main endpoint was overall survival. The effect of treatment schedule for each sex was explored using Cox proportional hazard regressions and multivariate analyses.

Findings: There was no sex differences in overall survival as a function of schedule in the population as a whole. However, overall survival was improved in males on chronoFLO as compared to CONV ($p = 0.009$), with respective median values of 20.8 [95 % CL, 18.7 to 22.9] and 17.5 months [16.1 to 18.8]. Conversely, median survival was 16.6 months [13.9 to 19.3] on chronoFLO and 18.4 months [6.6 to 20.2] on CONV in females ($p = 0.012$). The sex*schedule interaction was a strong predictive factor of optimal treatment schedule, with Hazard Ratios of 1.4 [1.05-1.86] for progression-free survival ($p = 0.021$) and 1.59 [1.30-1.75] for overall survival ($p = 0.002$) in multivariate analyses.

Conclusions: This meta-analysis shows that males lived significantly longer on chronomodulated rather than on conventional chemotherapy. Noteworthy the administration times in chronoFLO were selected according to circadian experiments in male mice and translational circadian studies in male patients. Specific studies should now address the issue of optimal circadian timing of chemotherapy in females. The current chronoFLO schedule should be recommended as first line treatment for males with metastatic colorectal cancers.

Introduction

The circadian timing system consists of a network of endogenous molecular clocks which generate about 24-h oscillations in each cell and are coordinated by a hypothalamic pacemaker¹. The circadian timing system gates cell division thereby regulating apoptosis and DNA repair as well as several signaling and metabolic pathways relevant for cancer processes and their treatments². The molecular clock is made up of interwoven transcription/translation regulatory loops involving fifteen clock genes and rhythmically controls cellular metabolism and proliferation. Molecular clocks are effectively coordinated through an array of physiological rhythms such as rest-activity, core body temperature, hormonal secretion and sympathetic/parasympathetic tone, which are synchronized by the hypothalamic pacemaker³. Both experimental and clinical evidence support an important role for circadian disruption in carcinogenesis and cancer progression, while circadian rhythm regulation enhances cancer inhibition⁴⁻⁶. Circadian timing of chemotherapy administration modifies its tolerability 2- to 10-fold, as shown for 40 anticancer drugs in experimental models². Strikingly, optimal antitumor efficacy usually results from drug delivery at the circadian stage when it achieves best tolerability and least disruption of the circadian timing system². The mechanisms responsible for this observation involve circadian changes in enzymatic activities and/or gene expression responsible for drug transport, bioactivation, detoxification, and pharmacodynamics². More specifically, several key rhythmic determinants of 5-fluorouracil and oxaliplatin tolerability peak 12 h apart both in nocturnally active rodents and in diurnally active humans. These findings led to test the effectiveness of chronomodulated chemotherapy, in an attempt to improve outcome and reduce toxicity in cancer patients. Phase I, II and III clinical trials tested the circadian timing hypothesis in patients with metastatic colorectal cancer². Three international randomized Phase III trials assessed whether the efficacy of first line 5-Fluorouracil - Leucovorin (5-FU-LV) and Oxaliplatin would benefit from a chronomodulated administration (ChronoFLO), as compared to conventional delivery (CONV), either as a constant rate flat infusion (flatFLO) or as FOLFOX2⁷⁻⁹. In the first two trials, ChronoFLO significantly improved objective response rate, when compared to CONV^{7,8}. Prolongation of survival was found in the first but not in the subsequent two studies⁷⁻⁹. The third trial revealed that ChronoFLO significantly improved survival as compared to FOLFOX in males, while an opposite effect was found in females⁹. The sex*schedule interaction was statistically validated in multivariate analyses for both progression-free survival and overall survival in this trial⁹. This finding led us to hypothesize that male and female patients with metastatic colorectal cancer responded differently to the circadian timing of 5-FU- and Oxaliplatin-based therapy. It is noteworthy that male mice were used for most of the preclinical chronotherapeutic studies of 5-FU and Oxaliplatin that were the basis for the chronomodulated schedule used in subsequent clinical trials². Moreover, male patients were mainly involved in the human translational studies that supported the concept of 5-FU and Oxaliplatin chronomodulation^{2,10}.

Here we update the individual data of the patients included in the three aforementioned international Phase III trials involving chronotherapy. We perform a metaanalysis in order to establish whether sex is a critical determinant of the optimal schedule of the reference three-drug chemotherapy against colorectal cancer.

Patients and methods

Study design

ChronoFLO involved chronomodulated infusions of 5-FU-LV from 2215 to 0945 hours with a peak at 0400 hours, and oxaliplatin from 1015 to 2145 hours with a peak at 1600 hours. In the first two trials (T1 and T2), patients were randomly assigned to receive either ChronoFLO or a flat infusion of the same three drugs (CONV) over 5 days every 3 weeks^{7,8}. In the third trial (T3), patients received either ChronoFLO over 4 days or FOLFOX2 every 2 weeks⁹. FOLFOX2 consisted of oxaliplatin and LV as a 2-hour infusion on day 1 and LV only on day 2, starting between 0900 and 1600 hours. The FU infusion was delivered at a constant rate for 22 hours on days 1 and 2⁹. In T3, intra-patient dose escalation was planned in both arms for 5-Fluorouracil, in order to treat each patient near individual maximum dose intensity.

The meta-analysis was conducted using individual patient data. Dates of progression and death were verified and updated for each patient. Follow up was terminated at 9 years after inclusion of the 1st patient in each trial.

Statistical methods

Individual data of all randomly assigned patients were included in the pooled analysis. Overall survival (OS) was defined as the time from date of randomization to the date of death from any cause. Patients lost to follow up before 9 years were censored on the date of their last visit. Progression-free survival (PFS) was defined as the time from randomization to progression or death whichever came first. Patients lost to follow-up or those with no date of progression recorded were considered as progression-free and censored at the date of last follow-up. Objective response (OR) was assessed every third or fourth treatment course according to trial specification, and defined according to WHO criteria.

Kaplan-Meier survival curves were plotted and Log Rank tests were performed to compare survival on ChronoFLO and CONV, in the whole population and separately in males and females. Response rates were compared according to schedule and sex using two-sided Chi-square test. The hazard ratios of an earlier death were represented for each variable and treatment schedule using Forrest plots. For PFS and OS, the relative benefit of treatment was explored using a trial-stratified Cox proportional hazard regression model, with a forward selection procedure. Logistic regression was used to explain differences in response rates, using a model with a trial specific intercept. Homogeneity between trials regarding the interaction between sex and treatment was investigated using Cochran's Q statistics, by testing the equality of the hazard ratios for PFS and OS, and of the odds ratios for the response rate. Candidate prognostic and predictive factors of OS, PFS and response were selected for multivariate analyses, based on statistical validation with $p = 0.10$ in univariate analysis. The effects of sex,

schedule, and their interaction were adjusted for each clinical prognostic factor selected upon univariate analysis. The final model included treatment schedule and sex with other factors added through a forward selection procedure. The 95% confidence intervals of Hazard Ratios (HR) for sex*treatment interaction and other prognostic factors were computed in the Cox models. The 95% confidence intervals of odds ratios (OR) for sex*treatment interaction and other prognostic factors were also computed in the logistic regression. All tests were two-sided. P-values < 0.05 were considered as statistically significant. All analyses were performed using SPSS 16.0. Forrest plots were drawn using R 2.12.2. The current report abides by the guidelines of the PRISMA statement ⁴⁴.

Results

Patient characteristics

From 1990 to 2003, 842 patients with metastatic colorectal cancer, (345 females and 497 males), were registered in one of three multicenter randomized trials, each one involving 8 to 36 centers in three to ten countries. Overall, ChronoFLO was administered to 180 female and 240 male patients, while CONV was given to 165 female and 257 male patients. The main clinical characteristics were similar in male and female patients on each treatment schedule, despite minor and non significant imbalances. The overall patient population had poor prognostic factors (Table 1). Patients received a median of 9 courses of protocol treatment. Median follow up was 93 months (67-108). The updated results of each trial revealed consistent improvements for all three efficacy endpoints in males on chronoFLO as compared to CONV. In contrast, female displayed inconsistent benefit from CONV vs. chronoFLO across the three trials (Supplementary Table 1).

Association between antitumor efficacy, treatment schedule and gender

Irrespective of sex, overall survival (OS) was similar for both treatment schedules, the median being 18.7 months [17.2 - 20.1] on chronoFLO and 17.6 months [16.6 - 18.6] on CONV (logrank $p = 0.66$). PFS did not differ between patients on chronoFLO or CONV, the median being 8.6 months [7.9 - 9.3] and 8.1 months [7.4 - 8.9] respectively (logrank $p = 0.92$). Objective response rate was 46% on chronoFLO and 39.8% on CONV ($p = 0.07$).

Interactions between sex and treatment schedule were investigated using multivariate Cox models for each survival endpoint, and a multivariate logistic model for response rate. No other interaction was introduced in the multivariate model, since no differential treatment effect was found in each category of any other clinical factor. The final models showed that sex*treatment interaction significantly modified OS, PFS and response rate. Thus, regarding OS, the Hazard Ratio (HR) of ChronoFLO4 relative to CONV was 1.32 in females ($p=0.018$), while this HR was 0.63 in males ($p=0.002$). Similar figures were found for the HR corresponding to PFS and, to a lesser extent, for the odds ratio related to response rate (Table 2). Other factors found to be significantly prognostic included performance status, number of metastatic sites, and percent liver invasion by tumor. This latter factor was only prognostic for OS (Table 2).

Homogeneity tests confirmed the consistent significant interaction between gender and treatment for OS, PFS and response rate among the three trials and the meta-analysis (Figure 1A).

The significant interaction terms found for each endpoint revealed a differential effect of schedule on outcomes in males and females, which was then modeled separately (supplementary Tables 2 and 3).

Effect of infusional schedule on antitumor efficacy according to gender

In males, OS was significantly prolonged on chronoFLO as compared to CONV (p from Logrank = 0.009) (Figure 2a); corresponding median values were 20.8 months [18.7 - 22.9] and 17.5 months [16.1 - 18.8]. Conversely, females on chronoFLO displayed a poorer OS as compared to CONV (p from Logrank = 0.012) (Figure 2b), with respective median values of 16.6 months [13.9 - 19.3] and 18.4 months [16.6 - 20.2]. At 5 and 9 years, 14.4% [9.9-18.9] and 9.2% [5.3 - 13.1] of males survived on chronoFLO as compared to 7.9% [4.6 - 11.2] and 3.8% [0.7 - 6.9] on CONV. At 5 and 9 years, 5.3% [2 - 8.6] and 4.4% [1.3 - 7.5] of females survived on chronoFLO compared to 15.6% [9.9 - 21.3] and 9.6% [3.9 - 15.3] on CONV.

Similar trends were found for both secondary efficacy endpoints (Supplementary Figure 1). A trend toward a better PFS was found in males on chronoFLO rather than CONV ($p = 0.088$), with respective median values of 9.3 months [8.4 - 10.3] and 8.4 months [7.5 - 9.3]. PFS was shorter in females on chronoFLO as compared to CONV ($p = 0.031$), with respective median values of 7.4 months [6.2 - 8.5] and 8.2 months [6.8 - 9.6]. The response rate was significantly higher in males on chronoFLO as compared to CONV (51.6% vs 37.8% respectively, Chi-square $p = 0.002$). In contrast the response rate in females was 38.3% on chronoFLO and 43% on CONV (Chi-square $p = 0.38$). These relationships were adjusted for the potential prognostic factors already considered in the whole population model.

Forrest plots and sex specific multivariate analyses confirmed that there was a lower risk of an earlier death on chronoFLO for males with a HR of 0.82 [0.68 - 0.99] ($p = 0.039$) and a higher risk for females with a HR of 1.36 [1.08 - 1.70] ($p = 0.009$) (Figure 2 c and supplementary Tables 2 and 3). Similar trends were found for PFS in males. Thus, the HR of an earlier progression on chronoFLO as compared with CONV was 0.86 [0.72 - 1.03] in males ($p = 0.11$) and 1.27 [1.02 - 1.58] in females ($p = 0.035$). For response rate, the odds ratio of an objective response on chronoFLO was significantly higher in males, being 0.55 [0.36 - 0.80] ($p = 0.002$), while no significant schedule effect was found in females.

Discussion

This meta-analysis of three international randomized trials demonstrated that sex of patient was the single significant predictor of the relative advantage of chronomodulated chemotherapy as the most effective delivery schedule of 5-Fluorouracil-Leucovorin-Oxaliplatin, the

main combination chemotherapy for metastatic colorectal cancer. Here, efficacy was assessed in 842 patients using the three most commonly used endpoints, i.e. overall survival, progression-free survival and tumor response rate. Using stringent methods, sex was shown to be the single robust predictor of the treatment schedule achieving best OR, best PFS and best OS, at univariate and multivariate or logistic analyses. For these three endpoints, males did better on chronoFLO than on CONV and females did better on CONV than on ChronoFLO, independently of all known baseline patient characteristics.

In 2011, first line chemotherapy of this disease still involves 5-Fluorouracil-Leucovorin, Oxaliplatin and/or Irinotecan¹²⁻¹⁴. Neither bevacizumab, cetuximab nor panitumumab improved survival when added to first-line FOLFOX either in unselected populations or in patients whose tumor displayed no *KRAS* mutation¹²⁻¹⁴. In contrast we show here that chronoFLO significantly enhanced median overall survival by 3.3 months as compared to conventional delivery of the same three drugs in males. This finding supports the chronomodulated administration of 5-fluorouracil-leucovorin-oxaliplatin as an important step for improving the outcome for male patients with metastatic colorectal cancer. The majority of patients alive at 9 years may be considered cured from a disease usually deemed as incurable¹⁵. Here, this was achieved in 9.2 % of the male patients treated with chronoFLO and 9.6 % of the females receiving a conventional infusion of the same three drugs.

Conventional therapy for localized colorectal cancer produced a better survival outcome in females as compared to males, in randomized trials involving over 1000 patients¹⁶. This finding is in good agreement with the better survival of females as compared to males on conventional chemotherapy in the current meta-analysis. However sex is seldom identified as an independent prognostic factor in clinical trials involving patients with colorectal cancer, since it is not usually examined as a possible source of interaction between the treatments under comparison. In a recent trial, panitumumab added to FOLFOX significantly prolonged progression-free survival in males but not in females with metastatic colorectal cancer carrying no *KRAS* mutation¹⁵. This finding highlights the need to examine treatment effects separately in males and in females, as recently advocated¹⁷. Different phenotypic and genotypic profiles were reported in male and female primary colorectal cancers. Females had more right sided tumors and a greater incidence of microsatellite instability (MSI), which was shown to predict for prolonged survival¹⁸. Males with mainly left-sided tumors did not benefit from adjuvant chemotherapy, while females with predominantly right-sided tumors did¹⁸. BRAF mutations were more frequent in stage II or III colon cancer in females as compared to males. BRAF mutation was an independent prognostic factor for survival outcome¹⁹. The presence of a functional EGFR polymorphism in colorectal cancer further predicted for a better survival in women and a worse one in men, as compared to patients of either sex with wild type EGFR tumor²⁰.

Experimental data show that the endocrine system regulates circadian drug metabolism as well as the circadian timing system itself. Androgens regulate the circuitry in the hypothalamic circadian pacemaker, with functional consequences for clock gene expression and behavioral responses to photic stimuli in mice²¹. However, our study did not reveal any significant trend as a function of age in females or in males, which suggests that sex hormone played little role in the sex effect shown here.

Excessive hematologic and non-hematologic toxicities have been reported in females on 5-Fluorouracil-based conventional chemotherapy^{22, 23}. This sex-dependent toxicity could result from differences in drug metabolism and detoxification. Thus, 5-Fluorouracil clearance as well as dihydropyrimidine dehydrogenase (DPYD) activity, its main determinant, were down-regulated in females as compared to males²⁴. Strikingly, however, the prediction of 5-Fluorouracil toxicity with *DPYD* gene polymorphism was robust and highly statistically significant in males but not in females, a finding which supports gender-specific toxicity mechanisms²². The severe toxicity associated with 5-Fluorouracil-based regimens affects females 20 to 50% more frequently than males. The occurrence of neutropenia during treatment was identified as an independent prognostic factor of longer survival in chemo-naïve patients on FOLFOX for metastatic colorectal cancer²⁵. This was confirmed in patients treated with FOLFOX2 in Trial 3 of this meta-analysis²⁶. In this same trial however, males on chronoFLO displayed both less neutropenia and better survival as compared to females⁸. These findings further highlight distinct schedule-dependent relations between toxicity and efficacy^{2, 26, 27}.

Chemotherapy can indeed disrupt the circadian timing system, thus impair the coordination of drug metabolism and pharmacodynamics over the 24 hour cycle². Indeed, 12 anticancer drugs dampened, phase shifted and/or suppressed physiological and molecular circadian rhythms as a function of dose and dosing time in experimental models². Transforming Growth Factor α , Interleukin 6 (IL-6) and Tumor Necrosis Factor α not only accelerate cellular proliferation but also impair circadian physiology and/or molecular clocks². High circulating levels of these three cytokines were associated with circadian disruption and poor survival in patients with metastatic colorectal cancer²⁸.

We hypothesize that the effect seen in our study stems from a sex specific response of the circadian timing system to cancer and/or its treatment. Both experimental and human data support a better stability of circadian rhythms in males as compared to females^{2, 27, 29}. Yet, the optimal chronomodulated schedule could also differ between male and female patients³⁰. There were over 2,000 rhythmic transcripts in males and females but only several hundred were common to males and females and peaked at a similar time of day. Both the circadian amplitude of melatonin secretion and that of core body temperature, as well as the entrainment properties of the circadian timing system also differed between male and female human subjects^{29, 30}.

In summary, this meta-analysis, using individual patient data from three chronotherapy trials, revealed sex as a robust determinant of the chemotherapy delivery schedule which offered best survival in patients with metastatic colorectal cancer. While ongoing research explores optimal chemotherapy timing in females, the chronotherapy schedule validated here should be recommended in male patients with metastatic colorectal cancer.

Legends to figures

Figure 1: Interaction between sex and treatment effects.. Results of equality of the hazard ratios for overall survival and progression free survival, and that of the odds ratios for response rate. Hazard ratios in each trial and in metaanalysis for (a) overall survival (p from Cochran Q test = 0.45), (b) progression-free survival (p = 0.90); (c) odds ratio for response rate (p = 0.50).

Figure 2: Sex differences in response to drug delivery schedule for overall survival. Kaplan Meier survival curves in males (a) and female (b), and Forrest plot of interaction between schedule and gender (c)

Table 1: Characteristics of the 842 patients included in the 3 randomized international trials

842 patients	Female		Male	
Characteristics	<i>Conv (%)</i>	<i>Chrono (%)</i>	<i>Conv (%)</i>	<i>Chrono (%)</i>
N of patients	165 (19.5)	180 (21)	257 (30.5)	240 (28.5)
Median age	60 (50-68)	59 (51-67)	62 (54-68)	62 (54-67)
Colon	126 (76)	141 (78.3)	183 (71.2)	173 (72.1)
Rectum	39 (24)	39 (21.7)	74 (28.8)	67 (27.9)
Performance Status				
0	69 (41.8)	80 (44.4)	134 (52.1)	122 (50.8)
1	75 (45.5)	78 (43.3%)	102 (39.7)	90 (37.5)
2	21 (12.7)	22 (12.2%)	21 (8.2)	28 (11.7)
Synchronous metastases	102 (61.8)	113 (62.8)	180 (70.0)	162 (67.5)
N of meta. sites				
1 (%)	93 (56.4)	93 (51.7)	136 (52.9)	128 (53.3)
2 (%)	48 (29.1)	67 (37.2)	84 (32.7)	81 (33.8)
≥3 (%)	24 (14.5)	20 (11.1)	37 (14.4)	31 (12.9)
Liver (%)	139 (84.2)	152 (84.4)	218 (84.8)	205 (85.4)
Lung (%)	55 (33.3)	66 (36.7)	95 (37)	87 (36.2)
Liver involvement				
<25%	72 (51.8)	85 (55.9)	111 (50.9)	120 (58.5)
≥25%	67 (48.2)	65 (42.8)	103 (47.2)	81 (39.5)
Unknown	0	2 (1.3)	4 (1.8)	4 (2.0)
Adjuvant Chemo. (%)	30 (18.2)	31 (17.2)	32 (12.5)	41 (17.1)
CEA , ng/mL				
≤10 (%)	38 (23 %)	50 (27.8%)	69 (26.8%)	62 (25.8%)

Table 2: Multivariate analysis of Overall Survival, Progression-Free Survival and Objective Response Rate. The reference group is female on conventional treatment

(a) Overall Survival			
Prognostic factor	Hazard ratio	IC 95%	p
Gender (M vs F)	1.12	0.95 - 1.45	0.13
Schedule (Chrono vs Conv)	1.32	1.05 – 1.65	0.016
Gender *Schedule	0.63	0.47 – 0.85	0.002
Performance Status (1& 2 vs 0)	1.51	1.30 - 1.75	<0.001
N of Sites (2 vs 1)	1.70	1.45 - 2.00	<0.001
(>2 vs 1)	1.90	1.52 - 2.37	<0.001
Percent liver involvement (<25% vs none)	0.75	0.61 - 0.93	0.009
(≥25% vs none)	1.25	1.01 - 1.55	0.044

(b) Progression-Free Survival			
Gender (M vs F)	1.03	0.84 - 1.27	0.75
Schedule (Chrono vs Conv)	1.23	0.99 - 1.53	0.061
Gender *Schedule	0.72	0.54 – 0.95	0.021
Performance Status (1&2 vs 0)	1.45	1.25 - 1.68	<0.001
N of Sites (2 vs 1)	1.72	1.42 - 2.08	<0.001
(>2 vs 1)	2.38	1.84 - 3.09	<0.001
Lung metastases	0.80	0.66 - 0.97	0.025
Percent liver involvement (<25% vs no involvement)	0.75	0.60 - 0.93	0.01
(≥25% vs no involvement)	1.03	0.83 - 1.29	0.77

(c) Objective Response Rate

Prognostic factor	Hazard ratio	IC 95%	p
Gender (M vs F)	1.30	0.86 - 1.96	0.21
Schedule (Chrono vs Conv)	1.23	0.79 - 1.91	0.36
Gender *Schedule	0.46	0.26 - 0.81	0.008
Performance Status (1& 2 vs 0)	1.47	1.10 - 1.98	0.01
N of Sites (2 vs 1)	1.35	0.98 - 1.85	0.065
(>2 vs 1)	1.87	1.19 - 2.95	0.007
Percent liver involvement (<25% vs none)	0.58	0.38 - 0.89	0.012
(≥25% vs none)	0.97	0.63 - 1.51	0.91
Trial (#2 vs #1)	1.27	0.75 - 2.16	0.37
(#3 vs #1)	1.03	0.65 - 1.65	0.88

Figure 1

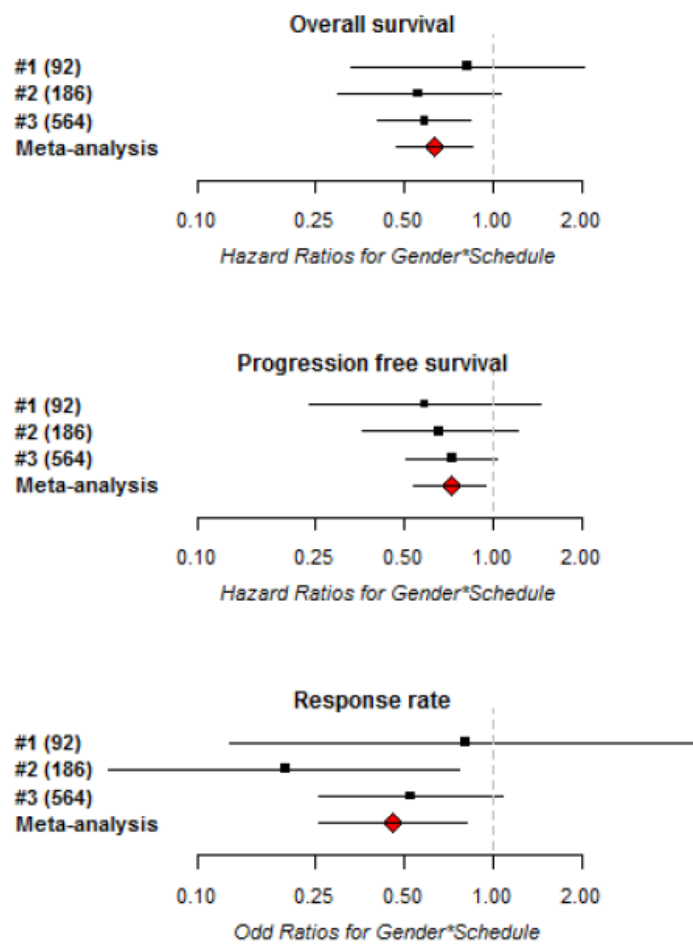
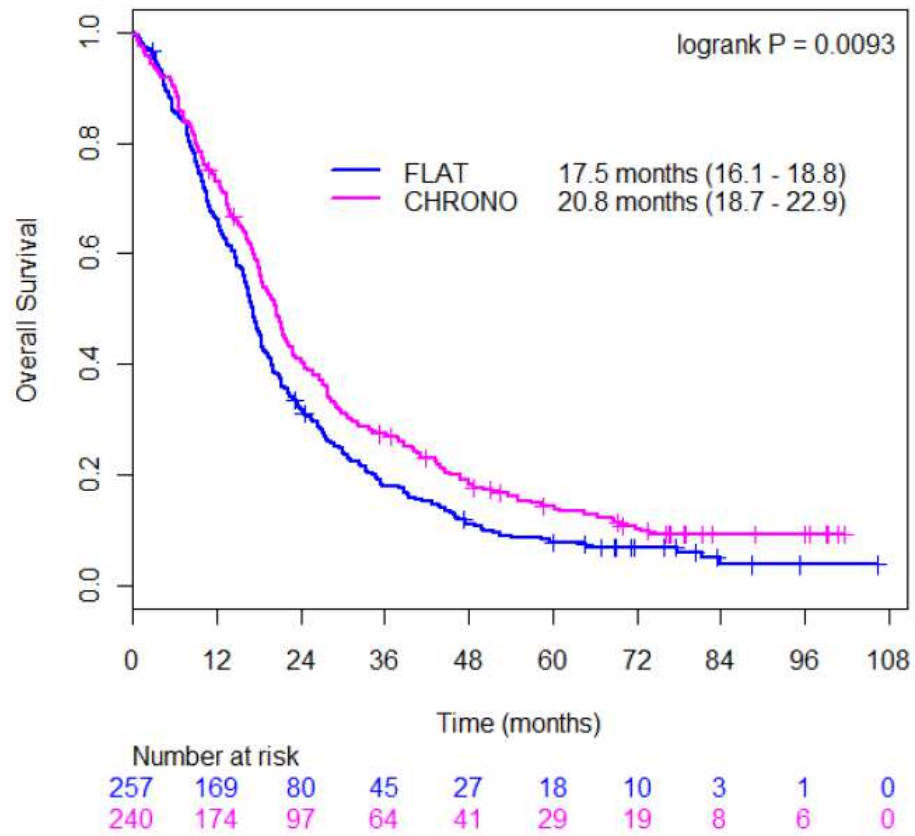
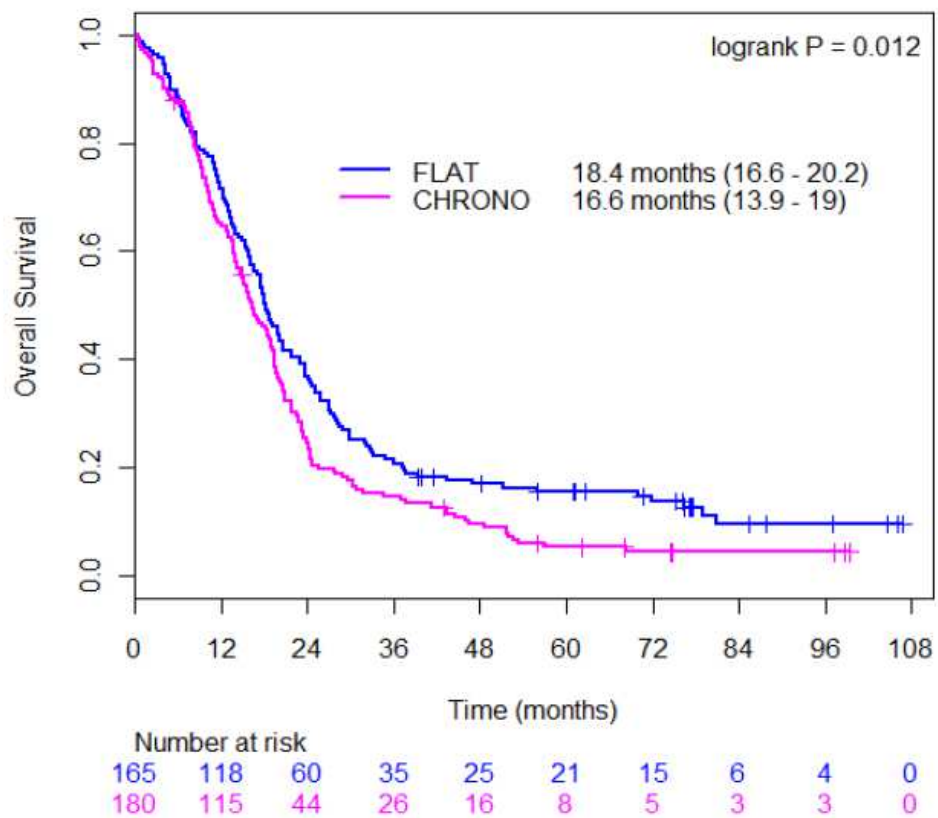


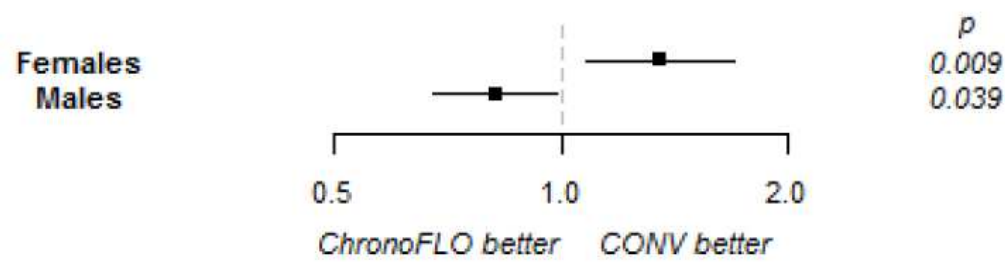
Figure 2
(a) Male



(b) Female



(c) Forrest Plot



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Supplementary Table 1: Updated results of the three randomized trials

		FEMALE		MALE		BOTH	
		<i>CONV</i>	<i>CHRONO FLO</i>	<i>CONV</i>	<i>CHRONO FLO</i>	<i>CONV</i>	<i>CHRONO FLO</i>
N OF PATIENTS	T1	20	25	27	20	47	45
	T2	33	41	60	52	93	93
	T3	112	114	170	168	282	282
MEDIAN OS (months) [95% CL]	T1	13.9 [12.8-14.9]	19.3 [16.7-22.0]	14.7 [14.3-15.2]	17.2 [11.4-23.]	14.6 [13.4-15.8]	19.1 [14.9-23.2]
	T2	17.9 [7.7-28.2]	14.0 [11.1-17.0]	15.3 [12.1-18.5]	17.3 [12.0-22.6]	16.8 [14.2-19.3]	15.4 [12.2-18.6]
	T3	19.0 [15.8-22.1]	16.2 [12.9-19.5]	18.1 [15.9-20.2]	21.0 [18.6-23.5]	18.5 [17.0-19.9]	19.3 [18.1-20.6]
	T1	7.6 [3.1-12.1]	8.4 [6.5-10.3]	7.0 [5.4-8.5]	10.4 [8.9-11.9]	7.0 [4.3-9.7]	9.8 [7.0-12.6]
	T2	6.9 [2.3-11.5]	8.0 [3.1-12.9]	7.3 [5.6-9.1]	8.8 [6.7-10.8]	7.3 [5.1-9.6]	8.7 [7.6-9.8]
	T3	8.1 [6.7-9.4]	6.9 [5.6-8.3]	8.7 [8.0-9.5]	9.1 [8.0-10.2]	8.5 [7.8-9.3]	8.1 [7.2-9.0]
MEDIAN PFS (months) [95% CL]	T1	30.0 [9.4-50.6]	48.0 [28.4-67.6]	33.3 [15.6-50.4]	60.0 [38.4-81.6]	31.9 [18.1-45.9]	53.3 [37.9-68.1]
	T2	30.3 [14.9-45.1]	34.1 [20.1-47.9]	28.3 [17.2-38.8]	63.5 [51.7-76.3]	29.0 [20.2-37.8]	50.5 [40.2-61.8]
	T3	49.1 [40.2-57.8]	37.7 [29.2-46.8]	41.8 [35.7-48.3]	47.0 [40.7-53.3]	44.7 [38.7-51.3]	43.3 [36.7-49.3]
	T1	30.0 [9.4-50.6]	48.0 [28.4-67.6]	33.3 [15.6-50.4]	60.0 [38.4-81.6]	31.9 [18.1-45.9]	53.3 [37.9-68.1]
	T2	30.3 [14.9-45.1]	34.1 [20.1-47.9]	28.3 [17.2-38.8]	63.5 [51.7-76.3]	29.0 [20.2-37.8]	50.5 [40.2-61.8]
	T3	49.1 [40.2-57.8]	37.7 [29.2-46.8]	41.8 [35.7-48.3]	47.0 [40.7-53.3]	44.7 [38.7-51.3]	43.3 [36.7-49.3]
ORR [95% CL]	T1	30.0 [9.4-50.6]	48.0 [28.4-67.6]	33.3 [15.6-50.4]	60.0 [38.4-81.6]	31.9 [18.1-45.9]	53.3 [37.9-68.1]
	T2	30.3 [14.9-45.1]	34.1 [20.1-47.9]	28.3 [17.2-38.8]	63.5 [51.7-76.3]	29.0 [20.2-37.8]	50.5 [40.2-61.8]
	T3	49.1 [40.2-57.8]	37.7 [29.2-46.8]	41.8 [35.7-48.3]	47.0 [40.7-53.3]	44.7 [38.7-51.3]	43.3 [36.7-49.3]

Supplementary Table 2 : Multivariate analysis of Overall Survival (a), Progression-Free Survival (b) and logistic regression of Objective Response Rate (c) in male patients

(a) Overall survival

Prognostic factor	Hazard ratio	IC 95%	p
Schedule (Chrono vs Conv)	0.82	0.68 – 0.99	0.039
Performance Status (1& 2 vs 0)	1.44	1.19 - 1.75	<0.001
N of Sites (2 vs 1)	1.93	1.56 - 2.39	<0.001
(>2 vs 1)	1.84	1.38 - 2.46	<0.001
Percent liver involvement (<25% vs none)	0.84	0.64 – 1.11	0.22
(≥25% vs none)	1.23	0.92 - 1.63	0.16

(b) Progression-free survival

Prognostic factor	Hazard ratio	IC 95%	p
Schedule (Chrono vs Conv)	0.86	0.72 – 1.03	0.11
Lung metastases	0.82	0.63 – 1.06	0.13
Performance Status (1& 2 vs 0)	1.46	1.21 - 1.76	<0.001
N of Sites (2 vs 1)	1.92	1.48 - 2.49	<0.001
(>2 vs 1)	2.40	1.70 - 3.39	<0.001
Percent liver involvement (<25% vs none)	0.85	0.63 – 1.15	0.29
(≥25% vs none)	1.08	0.80 - 1.45	0.62

(c) Response Rate

Prognostic factor	Hazard ratio	IC 95%	p
Schedule (Chrono vs Conv)	0.55	0.38 – 0.80	0.002
Performance Status (1& 2 vs 0)	1.58	1.08 – 2.32	0.019
N of Sites (2 vs 1)	1.13	0.75 – 1.71	0.56
(>2 vs 1)	1.58	0.88 - 2.83	0.12
Percent liver involvement (<25% vs none)	0.58	0.33 – 1.02	0.058
(≥25% vs none)	0.75	0.42 - 1.33	0.32
ETUDE (#2 vs #1)	1.07	0.52 – 2.18	0.85
(#3 vs #1)	1.14	0.60 – 2.16	0.69

Supplementary Table 3: Multivariate analysis of Overall Survival (a), Progression-Free Survival (b) and logistic regression of Objective Response Rate (c) in female patients

(a) Overall survival

Prognostic factor	Hazard ratio	IC 95%	p
Schedule (Chrono vs Conv)	1.36	1.08 – 1.70	0.009
Performance Status (1& 2 vs 0)	1.58	1.19 - 1.75	<0.001
N of Sites (2 vs 1)	1.48	1.15 – 1.90	0.002
(>2 vs 1)	2.13	1.49 – 3.05	<0.001
Percent liver involvement (<25% vs none)	0.61	0.44 – 0.86	0.004
(≥25% vs none)	1.25	0.89 - 1.75	0.19

(b) Progression-free survival

Prognostic factor	Hazard ratio	IC 95%	p
Schedule (Chrono vs Conv)	1.27	1.02 – 1.58	0.035
Lung metastases	0.77	0.58 – 1.04	0.087
Performance Status (1& 2 vs 0)	1.43	1.13 - 1.81	0.003
N of Sites (2 vs 1)	1.54	1.15 - 2.06	0.004
(>2 vs 1)	2.55	1.70 - 3.81	<0.001
Percent liver involvement (<25% vs none)	0.61	0.43 – 0.84	0.003
(≥25% vs none)	0.98	0.70 - 1.39	0.93

(c) Objective Response Rate

Prognostic factor	Hazard ratio	IC 95%	p
Schedule (Chrono vs Conv)	1.22	0.78 – 1.92	0.39
Performance Status (1& 2 vs 0)	1.40	0.87 – 2.23	0.16
N of Sites (2 vs 1)	1.75	1.06 – 2.89	0.03
(>2 vs 1)	2.57	1.22 – 5.43	0.013
Percent liver involvement (<25% vs none)	0.58	0.30 – 1.12	0.10
(≥25% vs none)	1.48	0.75 – 2.93	0.26
ETUDE (#2 vs #1)	1.71	0.76 – 3.83	0.20
(#3 vs #1)	0.93	0.47 – 1.85	0.84

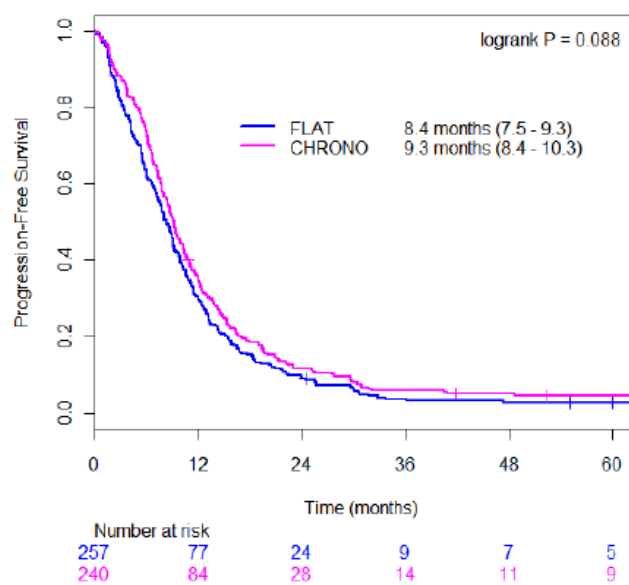
Legends to supplementary figures

Supplementary Figure 1: Relevance of drug delivery schedule for Progression Free Survival according to gender. Kaplan Meier survival curves in males (a) and female (b), and Forrest plot of interaction between schedule and gender (c)

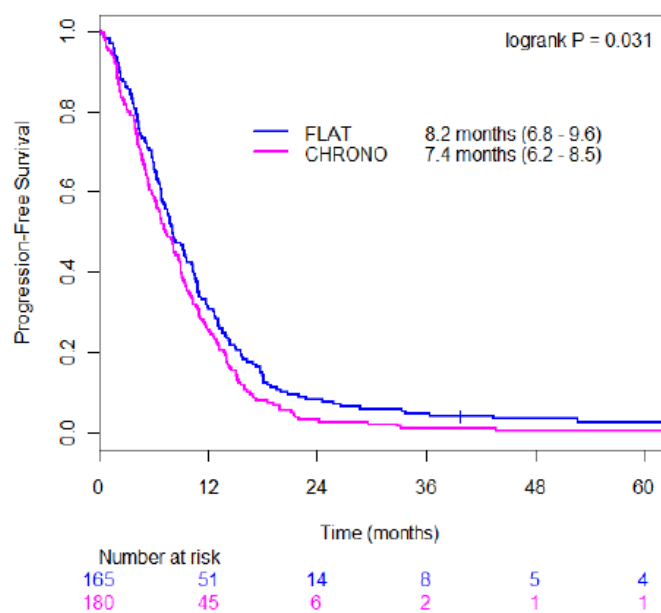
Supplementary Figure 2: Predictive factors of efficacy of 5-Fluorouracil-Leucovorin-Oxaliplatin, as assessed with overall survival. Hazard ratios and 95% confidence limits of relative effects of chronoFLO and CONV for each main patient characteristics. Note that gender was the single statistically significant predictor of chemotherapy efficacy for both variables

Supplementary Figure 1

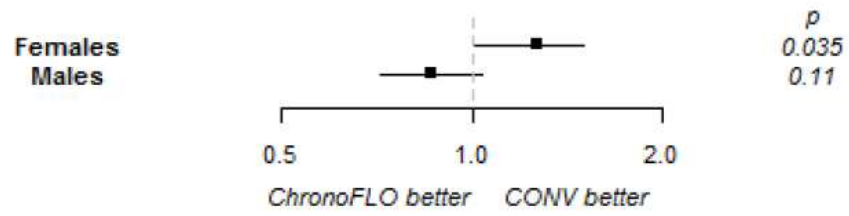
(a) Male



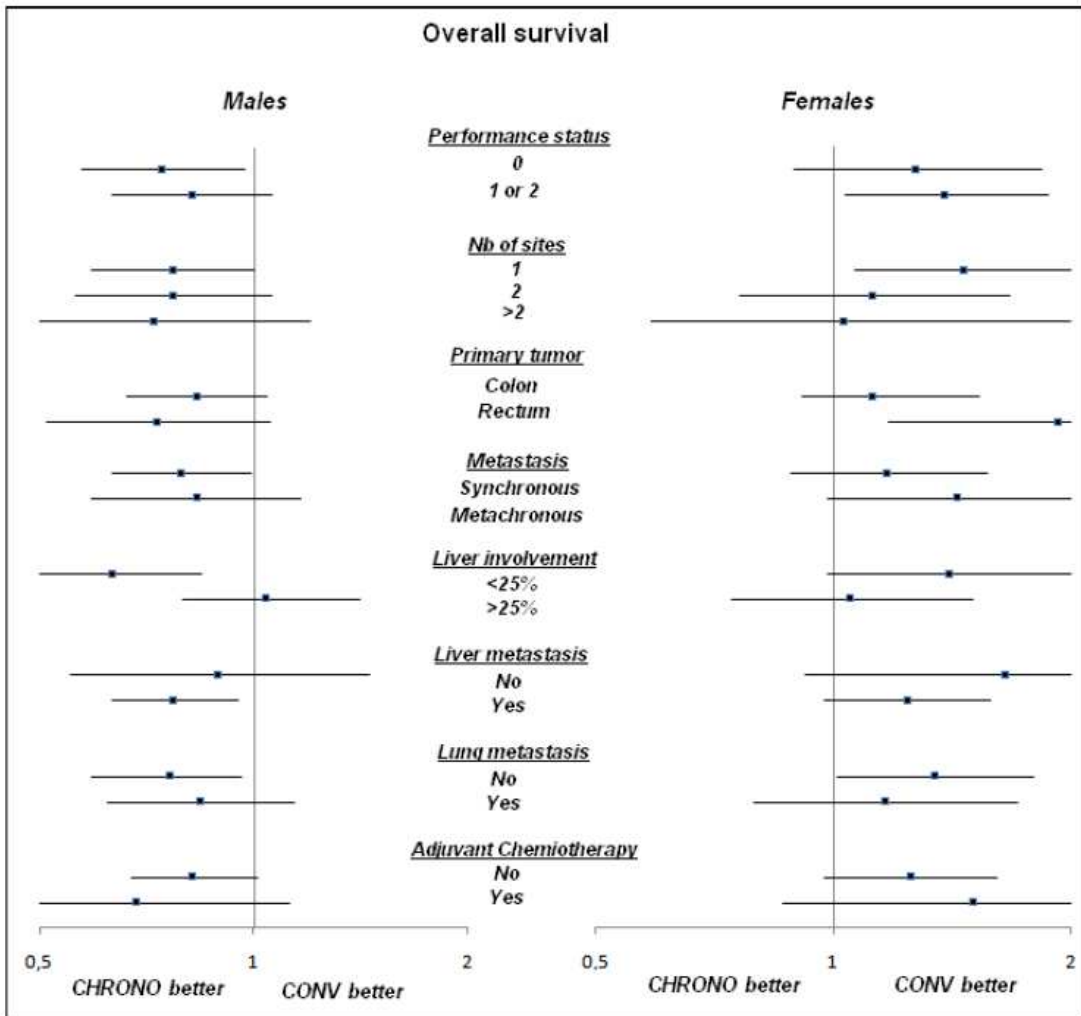
(b) Female



(c) Forrest Plot



Supplementary Figure 2



Prédiction de la survie par la survenue d'une neutropénie selon le schéma d'administration de l'association oxaliplatine-5-fluorouracile-acide folinique pour cancer colorectal métastatique dans un essai randomisé international (EORTC 05963)

Innominato PF, Giacchetti S, Moreau T, Smaaland R, Focan C, Bjarnason GA, Garufi C, Iacobelli S, Tampellini M, Tumolo S, Carvalho C, Karaboué A, Lévi F, pour le Groupe Internationale de Chronothérapie de l'ARTBC

Les horloges circadiennes contrôlent la prolifération cellulaire et le métabolisme des médicaments au cours des 24 heures. Pourtant, la chimiothérapie chronomodulée par 5-fluorouracile, leucovorine et oxaliplatine (chronoFLO4) n'a offert aucun avantage de survie par rapport au schéma FOLFOX2, sans stipulation d'horaires d'administration dans un essai randomisé international portant sur des patients atteints de cancer colorectal métastatique non antérieurement traité (EORTC05963). Nous avons fait l'hypothèse que le fait de traiter au voisinage des doses maximales tolérées de médicaments pourrait perturber les horloges circadiennes et nuire ainsi à l'efficacité du schéma chronoFLO4 mais non à celle du schéma FOLFOX2. Les patients avec données disponibles (N=556) ont été classés en trois groupes en fonction du pire grade de neutropénie observé au cours du traitement. Des modèles multivariés distincts avec des covariables dépendantes du temps ont été construits pour chaque schéma de traitement. L'incidence de neutropénie de tout grade était de 33% sous chronoFLO4 et de 61% sous FOLFOX2 ($p < 0,0001$), et celle de neutropénie de Grade 3-4 était de 7% sous chronoFLO4 et de 25% sous FOLFOX2 ($p < 0,0001$). La survenue de neutropénie était significativement plus fréquente chez les femmes que les hommes sous les deux schémas (FOLFOX2, $p = 0,003$; chronoFLO4, $p = 0,04$). La survie médiane sous FOLFOX2 était de 20,7 mois en cas de neutropénie G3-4 et de 12,5 mois en l'absence de neutropénie ($p < 0,0001$). Au contraire, sous chronoFLO4, les survies médianes correspondantes étaient de 13,7 et 19,4 mois ($p = 0,36$). L'analyse multivariée confirmait que la survenue d'une neutropénie sévère était un facteur pronostique indépendant de meilleure survie globale sous FOLFOX2 (HR=0,56, $p = 0,015$), mais de pire survie sous chronoFLO4 (HR=1,77, $p = 0,06$), avec un test d'interaction statistiquement significatif ($p < 0,0001$). La prédiction d'une meilleure survie chez les patients

neutropéniques sous FOLFOX2 est en faveur de l'administration des doses maximales tolérées de chimiothérapie conventionnelle. A l'inverse, la tendance montrée ici pour le schéma chronoFLO4 nous permet de proposer un nouveau paradigme d'optimisation conjointe de la tolérance hématologique et de l'efficacité de la chimiothérapie grâce à la personnalisation de la chronothérapie.

Prediction of Survival by Neutropenia According To Delivery Schedule of Oxaliplatin–5-Fluorouracil–Leucovorin for Metastatic Colorectal Cancer in a Randomized International Trial (EORTC 05963)

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Circadian clocks control cellular proliferation and drug metabolism over the 24 h. However, circadian chronomodulated chemotherapy with 5-fluorouracil, leucovorin, and oxaliplatin (chronoFLO4) offered no survival benefit as compared with the non-time-scheduled FOLFOX2, in an international randomized trial involving patients with previously untreated metastatic colorectal cancer (EORTC 05963). The authors hypothesized that treatment near maximum tolerated dose could disrupt circadian clocks thus impairing the efficacy of chronoFLO4 but not of FOLFOX2. Patients with available data (N=556) were categorized into three subgroups according to the worst grade (G) of neutropenia experienced during treatment. Distinct multivariate models with time-dependent covariates were constructed for each treatment schedule. Neutropenia incidence (all grades) was 33% on chronoFLO4 and 61% on FOLFOX2 ($p < .0001$), and G3–4 were 7% and 25%, respectively ($p < .0001$). Neutropenia was significantly more frequent in women than men on either schedule (FOLFOX2, $p = .003$; chronoFLO4, $p = .04$). Median survival was 20.7 mo in patients with G3–4 neutropenia versus 12.5 mo in neutropenia-free patients on FOLFOX2 ($p < .0001$). Corresponding figures were 13.7 and 19.4 mo, respectively, on chronoFLO4 ($p = .36$). Multivariate analysis confirmed occurrence of severe neutropenia independently predicted for better overall survival on FOLFOX2 (HR=0.56; $p = .015$), and worse survival on chronoFLO4 (HR=1.77, $p = .06$), with a significant interaction test ($p < .0001$). Prediction of better survival in neutropenic patients on FOLFOX2 supports the administration of conventional chemotherapy near maximum tolerated dose. The opposite trend shown here for chronoFLO4 supports the novel concept of jointly optimized hematologic tolerability and efficacy through personalized circadian-timed therapy. (Author correspondence: francis.levi@inserm.fr)

Keywords: Chemotherapy, Chronotherapy, Circadian, Colorectal cancer, Neutropenia, Prognostic factor

INTRODUCTION

Given the documented large interpatient variability in the catabolic and detoxification rates of anticancer drugs (Yang et al., 2010), cytotoxic chemotherapy-induced neutropenia

has been advocated as a biological indicator of proper dose intensity in individual patients (Felici et al., 2002; Kvinnsland, 1999). Indeed, the vast majority of studies has reported survival of neutropenic cancer patients was

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significantly longer than that of patients without such hematologic toxicity, as recently summarized in a meta-analysis (Shitara et al., 2010a). This was also the case for FOLFOX4 or mFOLFOX6 in an Asian cohort of 153 chemo-naïve patients with metastatic colorectal cancer, where neutropenia was positively associated with overall survival, independently of all other known prognostic factors (Shitara et al., 2009). In none of these studies was chemotherapy timing stipulated (Innominato et al., 2010; Lévi et al., 2010; Shitara et al., 2010a).

However, circadian timing was shown to significantly modify the extent of toxicity and efficacy of anticancer drugs, both in experimental models and cancer patients (Granda & Lévi, 2002; Innominato et al., 2010; Lévi & Schibler, 2007; Lévi et al., 2010). Indeed, the ~3-fold order of magnitude of inpatient circadian variability in 5-fluorouracil catabolism by dihydropyrimidine dehydrogenase matched that of interpatient variability of the same drug (Gamelin et al., 2008; Harris et al., 1990). Moreover, synchronous 24-h patterns have been shown for both tolerability and efficacy of most anticancer agents, including 5-fluorouracil and oxaliplatin (Granda et al., 2002; Peters et al., 1987). As a result, the administration of chemotherapy outside the optimal timing window can result both in excessive toxicity and poor antitumor efficacy (Lévi et al., 2010).

The joint optimization of tolerability and efficacy through proper circadian timing of anticancer drugs is further supported by proliferation data in human bone marrow and tumor tissue (Abrahamsen et al., 1997; Smaaland et al., 1991, 1992b, 1993, 2002). This concept challenges the current oncology principle that optimal antitumor effects result from treatment near maximum tolerated dose (Frei & Canellos, 1980). Indeed, experimental evidence has now documented that wrongly timed and/or too highly dosed anticancer drugs disrupt circadian physiology and/or the molecular clocks that constitute the circadian timing system (Lévi et al., 2010). This hierarchical network generates ~24-h (circadian) oscillations in cellular proliferation and metabolism (Lévi et al., 2007a, 2010; Lévi & Schibler, 2007; Liu et al., 2007; Sahar & Sassone-Corsi, 2009; Takahashi et al., 2008). Its disruption impairs the physiologic and molecular bases of treatment delivery according to circadian rhythms, so-called chronotherapy (Lévi et al., 2010).

In the current study, we investigated whether the relevance of chemotherapy-induced neutropenia for overall survival prediction also applied to patients treated with chronotherapy. Thus, in clinical oncology, toxicity of cancer chemotherapy is often positively associated with its efficacy (Frei & Canellos, 1980; Shitara et al., 2010a). This observation has given rise to the general oncologic practice of trying to treat each patient with therapy intensity titrated to near that individual's maximum tolerated dose. Generally positive results of this approach have led oncologists to the acceptance of the opinion that, in order to be effective, some level of cytotoxic drug toxicity must be accepted. However, chronotherapy work on both sides of

the Atlantic done over several decades has repeatedly and convincingly shown that when cytotoxic drugs are administered at their optimum times of day in order to avoid or minimize drug toxicity, this expected relationship between toxicity and efficacy is altered. In particular, higher doses can often be given with lower toxicity and concomitant increased or equal efficacy (Hrushesky, 1985; Lévi et al., 1990, 1997). The large randomized controlled multicenter clinical trial analyzed here confirms this dissociation as a unique reality in clinical oncology (Giacchetti et al., 2006). Herein, specifically, we first attempt to validate the positive association between neutropenia and survival in an independent cohort of patients receiving non-time-stipulated chemotherapy, with inpatient dose escalation up to maximum tolerated dose. The hypothesis that chronotherapy achieves best efficacy if tolerability is good is explored here clinically, using a data set obtained in a multicenter, randomized, controlled, phase III trial. The study compared chronomodulated (chronoflo4) versus conventional (FOLFOX2) flat infusion schedules combining oxaliplatin (L-OHP), 5-fluorouracil (5-FU), and leucovorin (LV), as first-line treatment for patients with metastatic colorectal cancer (EORTC 05963) (Giacchetti et al., 2006). Thus, in this trial, the patients received the same starting doses of the same drugs at the same frequency, though with pharmacologically different delivery schedules (Figure 1).

MATERIALS AND METHODS

Study Population

This study describes the results of an unplanned analysis of prospectively collected data from the international EORTC 05963 phase III trial, in previously untreated patients with metastatic colorectal cancer, randomized to receive either a conventional flat FOLFOX2 or a chronomodulated chronoflo4 schedule (Giacchetti et al., 2006). From October 1998 to February 2002, this trial enrolled 564 patients from 36 institutions in 10 countries (Giacchetti et al., 2006). The study, conducted in accordance with the Helsinki Declaration, was approved by the respective national ethics review boards involved, and all patients provided a signed informed consent (Portaluppi et al., 2010). Admission criteria in this trial included histologically proven, previously untreated colorectal adenocarcinoma with measurable and surgically unresectable metastatic lesions outside the brain, written informed consent according to national laws, adequate hematologic, renal, and hepatic functions, performance status (PS) ≤ 2 (World Health Organization [WHO] scale), age between 18 and 76 yrs, and no concomitant other cancer nor overt and uncontrolled cardiac, respiratory, chronic, or infectious diseases (Giacchetti et al., 2006).

Chemotherapy Schedules

FOLFOX2 involved the administration of L-OHP and LV as 2-h flat (non-varying-in-time constant) infusions on day 1, followed by constant-rate infusional 5-FU for 22 h. LV was

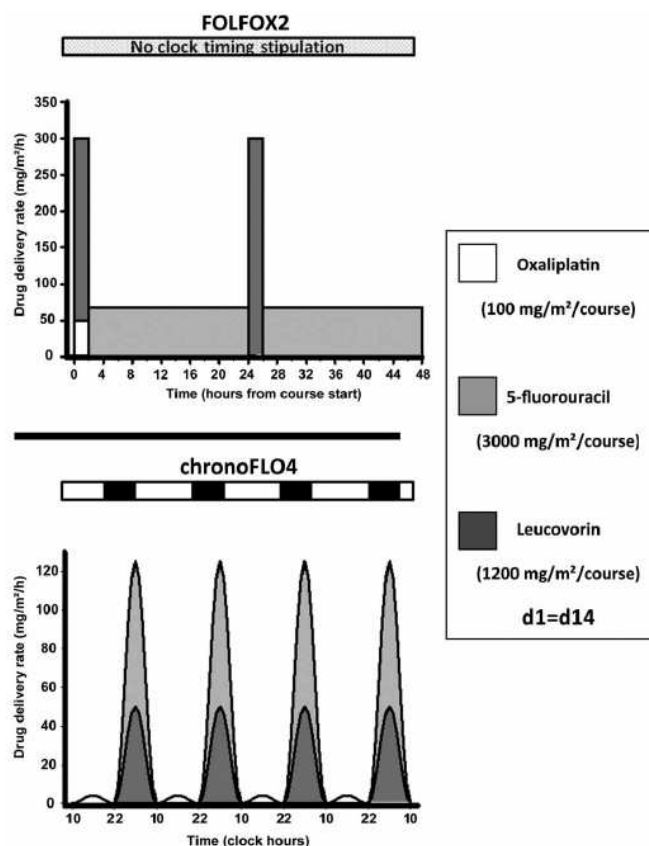


FIGURE 1. Chemotherapy delivery schedules. Top panel: conventional 2-day schedule FOLFOX2, with no stipulation of drug-administration time. Bottom panel: chronomodulated 4-day schedule chronoFLO4, with defined drug-administration time. Each schedule associated oxaliplatin (white), 5-fluorouracil (light gray), and leucovorin (dark gray) infusions were given fortnightly. 5-Fluorouracil dose was increased up to 20% if no Grade ≥ 2 toxicity occurred.

administered again on day 2 after 5-FU, and it was followed by constant-rate 5-FU infusion for 22 h (de Gramont et al., 1997; Giacchetti et al., 2006) (Figure 1, top panel).

ChronoFLO4 consisted of a 4-day course of alternating chronomodulated infusions of 5-FU-LV from 22:15 to 09:45 h, with a peak flow rate at 04:00 h, and L-OHP from 10:15 to 21:45 h, with a peak flow rate at 16:00 h (Giacchetti et al., 2006; Lévi et al., 1999) (Figure 1, bottom panel).

Courses were repeated in both arms every 14 days. Drug starting doses, 5-FU dose-escalation scheme, and drug-dose reductions followed the same guidelines in both arms. The starting doses per course were 100 mg/m² for L-OHP, 1200 mg/m² for LV, and 3000 mg/m² for 5-FU. This latter dose could be increased by 400 mg/m² on the second and by an additional 200 mg/m² on the third course, if no Grade ≥ 2 toxicity occurred. Dose

reductions (200 and 400 mg/m²/course for 5-FU and 10 and 20 mg/m²/course for L-OHP for Grades 3 and 4, respectively) were planned for patients experiencing clinical (diarrhea, stomatitis, hand-foot syndrome) and/or hematological (neutropenia, thrombocytopenia) toxicities. The subsequent course was delayed until every toxic event recovered to Grade 0 or 1 (National Cancer Institute Common Toxicity Criteria, version 2) (Giacchetti et al., 2006). Delivered dose intensities for L-OHP and 5-FU were calculated during the whole treatment duration as the actual total dose administered/m²/wk between day 1 of the first course until day 14 of the last course of chemotherapy received.

Neutropenia Evaluation

A complete hematologic assessment was performed fortnightly, prior to each chemotherapy administration.

Neutropenia was graded after each course according to the Common Terminology Criteria for Adverse Events, version 3.0. Each patient was classified into one of three categories according to the most severe grade (G) of neutropenia experienced throughout treatment: G0, none; G1-2, moderate; G3-4 or febrile neutropenia, severe. Primary prophylaxis with granulocyte-colony stimulating factors was not permitted.

Statistical Methods

The primary endpoint of this study was the association between neutropenia and overall survival, defined as the interval between the date of randomization and the date of death, due to any cause. Patients who were lost to follow-up ($n = 12$) before death were censored at the last contact date. The database was locked on November 9th, 2007; at this date, after a median follow-up of 87 mo (range: 68–108 mo), 507 events (89.9%) had occurred, and 45 patients were still alive. Given the rationale behind this study, the subsequent analyses were separately performed in each treatment arm. As preliminary analysis, the overall survival functions according to the severity of neutropenia were estimated and compared in the three groups of patients defined by the worst neutropenia experienced by each patient during follow-up. Kaplan-Meier's method and log-rank test were used for estimation and comparison, respectively. This approach was used simply for graphical representation but not for drawing conclusive results, since neutropenia was not a baseline feature and varied over time (Yamanaka et al., 2007). Therefore, the main analysis consisted of using Cox's regression models, including a time-dependent covariate (TDC) defined from the updated measurements of the chemotherapy-induced neutropenia. For each patient, the worst grade of neutropenia occurring between randomization and time $T > 0$ was defined as the value of the TDC at T (Shitara et al., 2009, 2010b; Yamanaka et al., 2007). The value of the variable for each individual patient, therefore, was not decreasing over time and could change over time according to the severity of neutropenia occurring by that time. The effect of the TDC on survival was adjusted for the following fixed covariates (Kohne et al., 2002; Sanoff et al., 2008): sex, age, PS at inclusion, number of metastatic sites, percentage of liver involvement with tumor, dose intensities of 5-FU and L-OHP, prior adjuvant chemotherapy, Dukes' stage at diagnosis, site of primary tumor, surgical resection of the primary tumor, prior surgery of metastases, baseline circulating leukocyte count, plasma alkaline phosphatase activity, and concentrations of carcinoembryonic antigen (CEA) and CA19.9.

The difference in the effect of worst neutropenia (as TDC) on overall survival between treatment arms was further explored by comparing the coefficients of the neutropenia variables obtained in the Cox models for each treatment arm. More precisely, the square of the difference between the natural logarithms of the hazard ratios (HRs) was divided by the sum of their variances;

under the null hypothesis of equality between the HRs, the above statistic is asymptotically distributed as a chi-square with 1 degree of freedom.

Neutropenia incidence and severity per patient was calculated in each treatment arm, and these rates were compared with a two-sided chi-square test. Comparisons among the three categories of patients (absent, moderate, or severe neutropenia) were performed using non-parametric Mann-Whitney or Jonckheere-Terpstra tests for continuous measurements and Pearson's chi-square or Fisher's exact two-sided tests for proportions of categorical variables. The prognostic value of neutropenia categories on objective response rate (WHO criteria) was assessed using a binary logistic regression model. Univariate Cox modeling was used to assess the effect of worst neutropenia experienced (as TDC) on progression-free survival. In order to avoid possible selection and lead-time bias due to higher risk of developing neutropenia according to the increasing number of cycles of chemotherapy received (Yamanaka et al., 2007), further sensitivity analysis was performed with a landmark that restricted neutropenia data for patient categorization to the first eight courses (Shitara et al., 2009). This analysis only included patients receiving at least eight treatment courses. The worst neutropenia encountered during the initial eight courses of chemotherapy was considered as a fixed covariate. Survival time was then computed from the completion of the eighth treatment course. Exploratory subgroup analyses were performed according to sex and baseline PS, in each treatment arm separately, to test the prognostic value of worst neutropenia (as TDC) on overall survival. Finally, associations between randomized treatment and progression-free survival, objective response rate, and overall survival were explored in the three categories of most severe neutropenia experienced (absent, moderate, or severe), using univariate Cox or binary logistic regressions. The threshold for statistical significance was set at $p < .05$.

All analyses were performed using PASW Statistics 18 software (SPSS, Chicago, IL, USA).

RESULTS

Neutropenia Incidence and Patient Features

Of the 564 patients randomized, 556 (98.6%) are included in the current analysis (279 in the FOLFOX2 arm and 277 in the chronoFLO4 arm). Six of the eight remaining patients did not start allocated treatment, whereas two received a single course without any available data on hematological toxicity. Nearly twice as many patients experienced neutropenia on FOLFOX2 ($n = 168$, 60.6%) than chronoFLO4 ($n = 91$, 32.6%) ($p < .0001$). Moderate (G1-2) or severe (G3-4) neutropenia occurred, respectively, in 119 (42.7%) and 69 (24.7%) patients on FOLFOX2 and in 91 (32.9%) and 18 (6.5%) patients on chronoFLO4 ($p < .0001$) (Figure 2A). The most severe episode of neutropenia occurred over the four initial courses for 20.2% of the patients, and over the eight

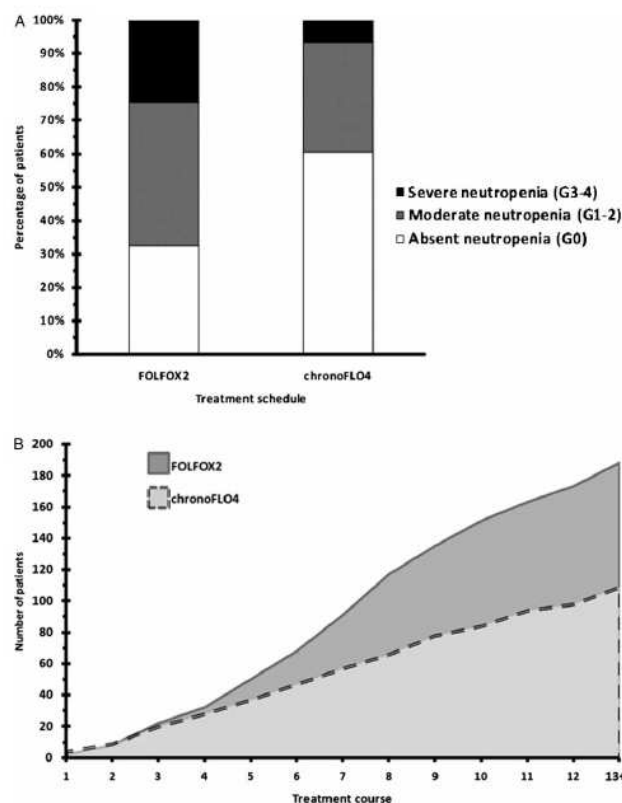


FIGURE 2. (A) Distribution of patients according to the most severe neutropenia experienced in each treatment arm. White boxes: no neutropenia (G0). Gray boxes: moderate neutropenia (G1-2). Black boxes: severe neutropenia (G3-4). (B) Treatment cycle corresponding to the occurrence of the first worst neutropenia episode, in each treatment arm. Continuous line: FOLFOX2. Dashed line: chronoFLO4.

initial ones for 61.6% of the patients. Only 7.4% of the patients displayed worst neutropenia after the 12th course (Figure 2B). Except for the very first course, moderate or severe neutropenia consistently occurred more frequently in patients on FOLFOX2 than chronoFLO4 (Figure 2B). Neutropenia occurred more frequently in females than males, both on FOLFOX2 (77.5% vs. 60.7%, respectively; $p = .003$) and on chronoFLO4 (46.5% vs. 34.4%, respectively; $p = .04$). Similarly, a trend toward a higher incidence of G3-4 neutropenia was found in women as compared to men, both on FOLFOX2 (30.6% vs. 20.8%; $p = .06$) and on chronoFLO4 (9.6% vs. 4.3%; $p = .08$). Irrespective of sex, neutropenia was more frequent and more severe in patients receiving FOLFOX2 than chronoFLO4 ($p < .0001$).

Actual Dose Intensities

The dose intensities of L-OHP and 5-FU varied significantly according to neutropenia category in each treatment arm ($p \leq .011$) (Table 1 and Figure 3). Two-by-two comparisons

with a threshold p value of .001 are tagged in Figure 3. Moderate or severe neutropenia was associated with comparable dose intensities in both schedules (Table 1 and Figure 3). Conversely, in neutropenia-free patients, the actual dose intensities of L-OHP and 5-FU were lower on chronoFLO4 as compared with FOLFOX2 ($p < .0001$ and $p = .004$, respectively) (Table 1 and Figure 3).

Prognostic Value of Neutropenia on Objective Response and Progression-Free Survival

Objective response rate varied significantly according to the severity of the worst neutropenia in each treatment arm. Thus, response rate was lowest in the neutropenia-free patients than the neutropenic ones, both on FOLFOX2 ($p < .0001$) and on chronoFLO4 ($p = .004$) (Table 1). The occurrence of neutropenia on FOLFOX2 was significantly associated with longer progression-free survival (Table 1) ($p = .034$). Conversely, an opposite trend was noted for chronoFLO4 ($p = .061$), with patients experiencing severe neutropenia displaying a

TABLE 1. Patient characteristics

Characteristic		FOLFOX2				chronofLO4			
		G0 (absent) (n = 91)	G1-2 (moderate) (n = 119)	G3-4 (severe) (n = 69)	All (n = 279)	G0 (absent) (n = 168)	G1-2 (moderate) (n = 91)	G3-4 (severe) (n = 18)	All (n = 277)
Age (yrs)	Median	62	62	64	62	62	62	60	62
	Range	33-76	32-76	37-75	32-76	24-76	22-76	37-71	22-76
Sex (%)	Female/Male	28/73	44/56	49/51	40/60	36/64	46/54	61/39	41/59
PS (%)	0/1/2	41/44/16	54/40/7	54/41/6	50/41/9	49/40/11	48/41/11	33/50/17	48/41/12
# metastatic sites (%)	1/2 / ≥ 3	45/35/20	53/31/16	54/30/16	51/32/17	48/36/17	58/36/6	50/33/17	51/36/13
% liver tumor involvement	None/ ≤ 25/25%	17/37/46	8/50/43	23/48/29	14/45/41	14/44/42	15/58/26	11/44/44	14/49/37
Prior adjuvant chemotherapy (%)	Yes	22	12	19	17	16	22	28	18
Synchronous metastases (%)	Yes	73	81	64	74	76	73	72	75
primary tumor Site (%)	Colon/rectum	79/21	76/24	71/29	76/24	79/21	72/28	78/22	77/23
Prior surgery: primary tumor (%)	Yes	86	87	84	86	89	88	89	89
Prior surgery: metastases (%)	Yes	3	7	4	5	5	3	17	5
Baseline WBC (%)	10.0 × 10 ⁹ /L	41	19	26	28	31	11	28	24
Baseline ALP (%)	≥300 IU/L	34	25	19	27	33	23	22	29
Baseline CEA (%)	≥10 ng/mL	66	70	62	67	68	57	78	65
Baseline CA19.9 (%)	≥37 IU/L	50	48	45	48	48	42	44	46

Cycles: total (%)	Median	8	12	11	10	9	11	8	10
	1st and 3rd quartiles	4-10	9-15	8-12	8-12	4-12	8-13	6-11	6-12
	Range	1-21	4-40	2-49	1-49	1-44	1-28	2-22	1-44
Dose intensity of oxaliplatin (mg/m ² /wk)	Median	45.3	38.6	35.9	40.1	40.8	38.8	33.2	39.4
	1st and 3rd quartiles	39.3-50.6	32.2-44.8	31.6-41.9	33.8-46.4	33.3-46.6	32.7-44.0	27.3-38.9	32.5-44.7
	Range	11.7-55.7	11.2-51.7	9.4-48.3	9.4-55.7	5.5-60.9	10.8-53.9	4.4-51.2	4.4-60.9
Dose intensity of 5-fluorouracil (g/m ² /wk)	Median	1.51	1.41	1.25	1.41	1.41	1.36	1.17	1.38
	1st and 3rd quartiles	1.36-1.69	1.25-1.56	1.11-1.42	1.24-1.60	1.22-1.61	1.15-1.51	0.98-1.39	1.19-1.56
	Range	0.70-1.82	0.64-1.81	0.60-1.73	0.60-1.82	0-1.95	0.69-1.76	0.61-1.69	0-1.95
Objective response rate (%)	Value	16.5	60.5	56.5	45.2	36.3	58.2	44.4	44.0
	95% CI	8.2-25.8	52.2-69.8	44.7-69.3	38.7-51.3	29.7-42.3	27.2-48.8	20.9-67.1	37.7-50.3
Median time to progression (mo)	Value	4.5	9.7	9.7	8.5	7.7	9.3	9.2	8.3
	95% CI	2.9-6.1	8.5-10.9	8.6-10.8	7.8-9.3	6.8-8.7	7.8-10.8	4.5-13.9	7.4-9.2
Incidence of other G3-4 toxicities*	% of patients	55.6	75.6	70.1	67.8	68.3	75.6	82.4	71.5

Patient characteristics in each treatment arm according to the categories of the worst neutropenia experienced.
PS = performance status (WHO scale); WBC = white blood cells; ALP = alkaline phosphatases; IU = international units.
*Excluding leukopenia and neutropenia.

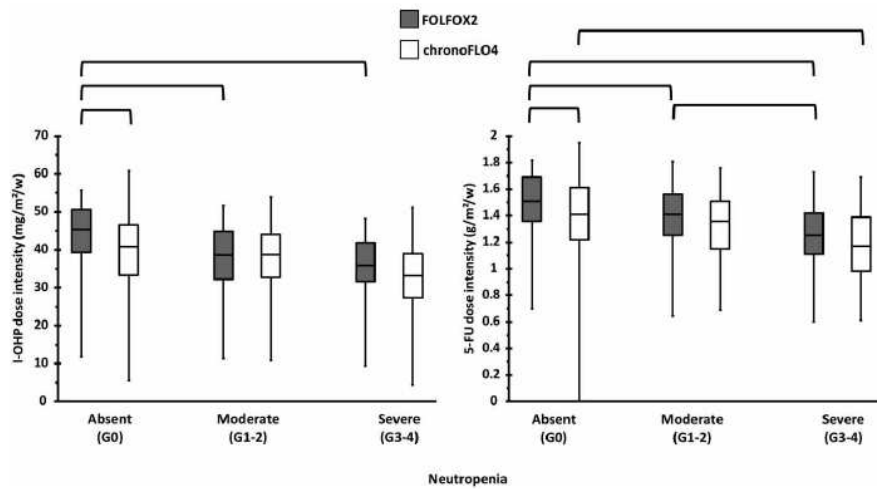


FIGURE 3. Boxplots representing the actual dose intensity of oxaliplatin (I-OHP, mg/m²/wk; left panel) and 5-fluorouracil (5-FU, g/m²/wk; right panel), according to the categories of worst neutropenia, in each treatment arm (FOLFOX2, grey boxes; chronoFLO4, white boxes). Boxes: 1st and 3rd quartiles; middle line: median; bars: range. Highly statistically significant ($p \leq .001$) two-by-two differences are tagged.

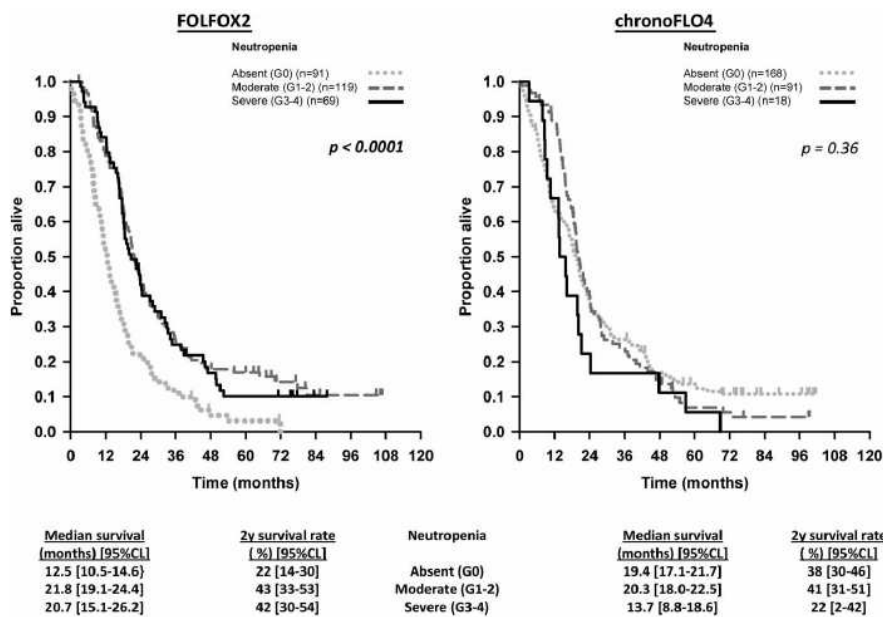


FIGURE 4. Overall survival curves in each treatment arm (FOLFOX2, left panel; chronoFLO4, right panel) according to the occurrence of the most severe neutropenia. Dotted light gray lines: no neutropenia (G0). Dashed darker gray lines: moderate neutropenia (G1-2). Solid black lines: severe neutropenia (G3-4). p values derived from log-rank test. This represents the graphical display of preliminary analyses.

TABLE 2. Cox proportional hazard models for overall survival

Treatment schedule		FOLF0X2				ChronoFLO4			
Parameter		Univariate		Multivariate with TDC		Univariate		Multivariate with TDC	
		HR	p	HR	p	HR	p	HR	p
Neutropenia (TDC)	Absent (G0)	1	<0.0001	1	0.04	1	0.025	1	0.07
	Moderate (G2-3)	0.58 [0.43-0.78]		0.67 [0.44-1.00]		1.17 [0.89-1.54]		1.36 [0.96-1.92]	
	Severe (G3-4)	0.57 [0.40-0.79]		0.56 [0.35-0.90]		1.86 [1.18-2.92]		1.77 [0.98-3.20]	
Sex	Female	1	0.065	1	0.32	1	<0.0001	1	0.07
	Male	1.28 [0.99-1.65]		1.20 [0.84-1.71]		0.63 [0.49-0.81]		0.73 [0.52-1.03]	
Baseline PS	0	1	<0.0001	1	0.009	1	<0.0001	1	0.43
	1	1.56 [1.20-2.03]		1.13 [0.79-1.61]		1.70 [1.30-2.23]		1.29 [0.87-1.91]	
	2	4.80 [3.07-7.51]		2.58 [1.40-4.74]		1.63 [1.09-2.46]		1.23 [0.72-2.12]	
Number of metastatic sites	1	1	<0.0001	1	0.009	1	0.008	1	0.007
	2	1.87 [1.41-2.48]		1.46 [1.03-2.07]		1.47 [1.12-1.92]		1.73 [1.22-2.44]	
	≥3	2.18 [1.54-3.08]		1.96 [1.19-3.22]		1.56 [1.06-2.28]		1.50 [0.87-2.59]	
Percentage of liver involvement by tumor	None	1	0.049	1	0.35	1	0.006	1	0.23
	≤25%	0.93 [0.64-1.35]		0.68 [0.40-1.15]		0.80 [0.55-1.16]		0.67 [0.38-1.17]	
	>25%	1.29 [0.89-1.89]		0.73 [0.41-1.29]		1.24 [0.85-1.83]		0.84 [0.46-1.54]	
Surgery of primary tumor	Yes	0.66 [0.46-0.93]	0.017	0.75 [0.48-1.18]	0.22	0.86 [0.58-1.26]	0.43	1.15 [0.70-1.87]	0.59
Baseline WBC	≥10.0 × 10 ⁹ /L	1.86 [1.42-2.45]	<0.0001	1.82 [1.24-2.68]	0.002	1.45 [1.09-1.93]	0.037	0.93 [0.61-1.43]	0.74
Baseline ALP	≥300 IU/L	1.91 [1.44-2.53]	<0.0001	1.66 [1.11-2.49]	0.014	1.64 [1.24-2.16]	0.001	1.33 [0.91-1.97]	0.15
Baseline CA19.9	≥37 IU/L	1.71 [1.24-2.36]	0.001	1.46 [0.99-2.13]	0.055	1.94 [1.41-2.67]	<0.0001	1.51 [1.05-2.18]	0.027
Dose intensity of oxaliplatin (mg/m ² /wk)	Quantitative	1.05 [1.03-1.06]	<0.0001	1.05 [1.02-1.08]	0.002	1.04 [1.02-1.05]	<0.0001	1.07 [1.04-1.11]	<0.0001
Dose intensity of 5-fluorouracil (g/m ² /wk)	Quantitative	3.10 [1.81-5.29]	<0.0001	0.36 [0.13-1.05]	0.06	1.56 [0.94-2.59]	0.083	0.34 [0.12-1.01]	0.052

Univariate and multivariate Cox proportional hazard models for overall survival in each treatment arm. Neutropenia is a time-dependent covariate (TDC). Other adjustment factors, which were never significant, included age, prior adjuvant chemotherapy, Dukes' stage at diagnosis, site of primary tumor, prior surgery of metastases, and baseline CEA. HR = hazard ratio [95% confidence interval]; PS = performance status (WHO scale); WBC = white blood cells; ALP = alkaline phosphatases; IU: international units.

higher risk of earlier progression than neutropenia-free patients (HR: 1.71; 95% confidence interval [CI]: 1.08–2.71; $p = .023$).

Prognostic Value of Neutropenia on Overall Survival

Median (95% CI) overall survival was 18.5 (17.0–19.9) mo in the FOLFOX2 arm and 19.3 (18.1–20.6) mo in the chronoFLO4 arm.

Preliminary analysis showed that overall survival functions varied significantly according to neutropenia category in the FOLFOX2 arm ($p < .0001$), but not in the chronoFLO4 arm ($p = .36$) (Figure 4). Overall survival was similar in patients receiving FOLFOX2 with moderate or severe neutropenia (Figure 4 and Table 2).

Cox proportional hazard modeling with neutropenia as the time-dependent covariate (TDC) confirmed that the decreased risk of an earlier death conferred by occurrence of neutropenia (moderate or severe) on FOLFOX2 remained statistically significant following adjustment for other known prognostic factors ($p = .04$) (Table 2). On the contrary, although the risk of earlier death associated with the occurrence neutropenia (as TDC) on chronoFLO4 significantly increased when this toxicity arose ($p = .025$), multivariate Cox proportional hazard modeling found no significant prognostic value for neutropenia on this treatment modality, even though a consistently opposite trend to that observed for FOLFOX2 was found ($p = .07$) (Table 2), with patients with severe neutropenia on chronoFLO4 displaying poorest survival. Thus, the occurrence of severe neutropenia was independently associated with survival, yet with an opposite relative risk of an earlier death, according to treatment modality: a significantly lower risk on FOLFOX2 (HR: 0.56; 95% CI: 0.35–0.90; $p = .015$) and a nonsignificant trend toward a higher risk on chronoFLO4 (HR: 1.77; 95% CI: 0.98–3.20; $p = .06$). Indeed, highly significant difference in the effect of the occurrence of neutropenia between treatment arms was observed concerning both moderate and severe neutropenia (all $p < .001$). These results remained similar if the worst neutropenia observed for each patient was considered as a simple baseline prognostic covariate (not shown).

Subgroup analyses according to sex or baseline PS confirmed the better prognosis in patients with moderate or severe neutropenia on FOLFOX2 in both women and men and in each PS subgroup ($p \leq .18$; not shown). Conversely, patients with moderate or severe neutropenia on chronoFLO4 tended to display an increased risk of earlier death in each subgroup analyzed, although never with statistical significance ($p \geq .09$; not shown). Thus, the opposite trend of the association between neutropenia and overall survival according to the pharmacological schedule found in the whole cohort was recapitulated in every subgroup assessed.

The landmark analysis limited neutropenia data to the first eight courses, and included the 404 patients who received at least eight treatment courses (212 patients on FOLFOX2 and 192 patients on chronoFLO4). Thus, the

relative proportion of patients with moderate or severe neutropenia remained significantly higher on FOLFOX2 than chronoFLO4 ($p < .0001$). Furthermore, the survival of neutropenia-free patients was significantly shorter than that of patients with moderate or severe neutropenia on FOLFOX2 ($p = .051$). Conversely, neutropenia-free patients lived longer than the neutropenic ones on chronoFLO4 ($p = .002$). In this landmark group as well, the interaction between the randomized treatment schedule and worst neutropenia grade significantly influenced overall survival ($p = .005$); this association persisted independently from the other prognostic factors in a multivariate analysis ($p = .001$). Further exploratory analyses showed that the chances of obtaining a better outcome, i.e., higher objective response rate, longer progression-free survival, and longer survival, were significantly increased in neutropenia-free patients receiving chronoFLO4 and in severely neutropenic patients treated with FOLFOX2 (Figure 5).

DISCUSSION

This international study confirmed, in an independent cohort of patients, that the occurrence of chemotherapy-induced neutropenia is associated with improved survival. This was here the case for patients receiving conventional FOLFOX for previously untreated metastatic colorectal cancer (Shitara et al., 2009). The clinical trial design involved higher than usual starting doses and inpatient dose escalation so as to administer the maximum tolerated dose in each individual patient (Colucci et al., 2005; Cunningham et al., 2009; de Gramont et al., 2000; Giacchetti et al., 2006, 2000; Maindrault-Goebl et al., 2001). The timing of drug administration was not stipulated and varied both among patients and between courses for FOLFOX2 (Giacchetti et al., 2006; Innominato et al., 2010; Lévi et al., 2010). Conversely, chronoFLO4 delivered the three same drugs at defined times so as to take into account the circadian timing system (Giacchetti, 2002; Hrushesky & Bjarnason, 1993; Innominato et al., 2010; Lévi, 2002; Lévi & Schibler, 2007; Lévi et al., 2007b, 2010). This approach was based on data showing that predictable 24-h changes characterize the detoxification of anticancer drugs, as well as tissue-specific replication rates, especially in human bone marrow (Abrahamsen et al., 1997; Bjarnason & Jordan, 2002; Bjarnason et al., 1999, 2001; Buchi et al., 1991; Harris et al., 1990; Lévi & Schibler, 2007; Lévi et al., 2010; Smaaland et al., 1991, 1992a, 2002).

Circadian rhythms in therapeutic effects are generated by molecular clocks residing within each cell and coordinated by a hypothalamic pacemaker (Dibner et al., 2010; Lévi & Schibler, 2007; Lévi et al., 2010; Paschos et al., 2010). Wrongly timed or excessively dosed cancer medications, in turn, profoundly disrupt circadian physiology and molecular clocks in experimental models (Lévi et al., 2010). This results in severe alterations of the circadian

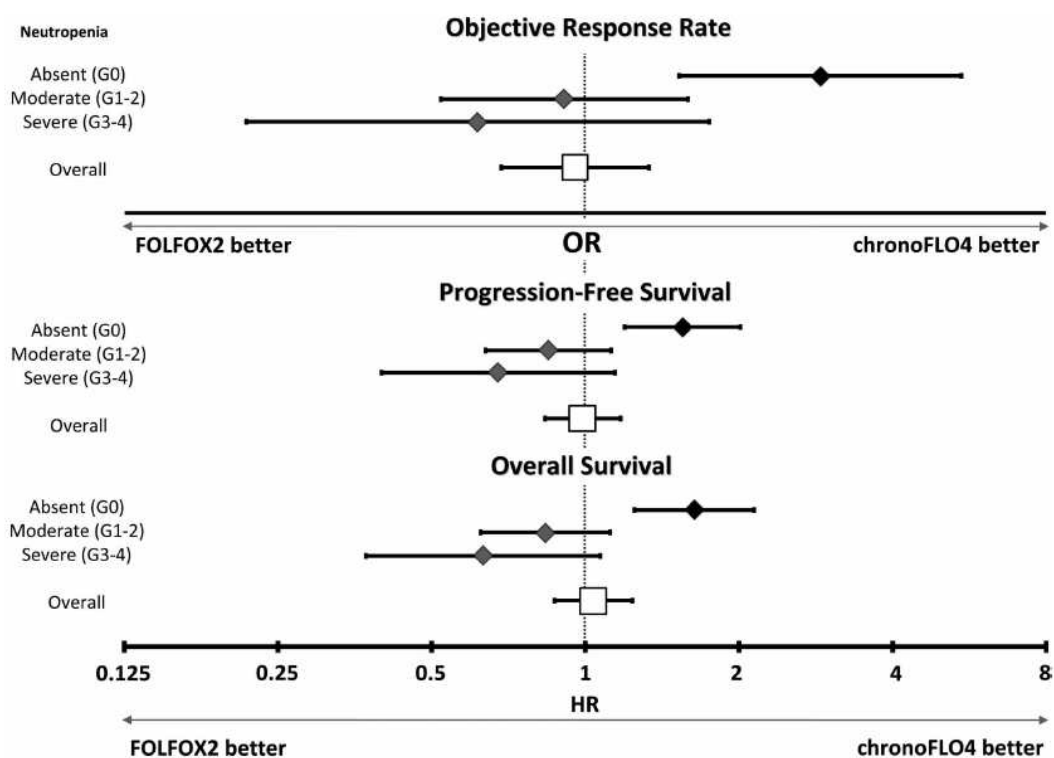


FIGURE 5. Forest plot of the effect of treatment in each subgroup of patients defined by the most severe neutropenia developed. OR = odds ratio; HR = hazard ratio.

coordination of both drug-detoxification pathways and cell-cycle gating. This circadian disruption interferes with the beneficial control exerted by the circadian timing system on tumor progression, and can concurrently hamper both treatment tolerability and anticancer efficacy (Filipski et al., 2002, 2004, 2005; Lévi et al., 2010). Indeed, robust circadian host physiology predicted for prolonged survival, independently of other known prognostic factors in patients with breast or colorectal metastatic cancer (Innominato et al., 2009; Mormont et al., 2000; Sephton et al., 2000). Furthermore, this concept contrasts chronotherapeutics with the principle that governs conventional cancer treatments: the larger the treatment toxicity, the better the antitumor efficacy (Frei & Canellos, 1980; Shitara et al., 2010a). However, 2 studies out of 13 reported no positive association between the occurrence of Grade 3–4 leukopenia and survival (Kim et al., 2010; Miyoshi et al., 2009; Shitara et al., 2010a). In our and most other prior studies, no difference in survival has been found between patients with moderate or severe neutropenia receiving non-time-stipulated chemotherapy (Figure 4) (Shitara et al., 2010a). These findings suggest that in comparing severe to absent or moderate neutropenia, one could blunt existing survival differences, mostly related to the occurrence and not the severity of neutropenia (Figure 4) (Shitara et al., 2010a). The current study provides the first clinical evidence of a substantial difference between non-time-stipulated and circadian-based chemotherapy for the toxicity-efficacy relationship; despite the same drug doses and course frequency being used, the overall observed outcomes were comparable (Giacchetti et al., 2006). Neutropenic patients on FOLFOX2 displayed best efficacy outcomes independently of other known prognostic factors, in accordance with prior reports (Shitara et al., 2009, 2010a; Yamanaka et al., 2007). Conversely, the occurrence of neutropenia with chronoFLO4 was not associated with any improvement in survival, and was even associated with impaired efficacy outcomes. Despite the low incidence of Grade 3 or 4 hematologic toxicity on chronoFLO4 (6.5%; Figure 2, Table 1), the negative trend that characterized the relationship between neutropenia and survival was almost statistically significant ($p = .07$; Table 2). Moreover, the earlier the occurrence of neutropenia on chronoFLO4, the poorer the survival on this schedule, according to the sensitivity analyses in the landmark group ($p = .002$). The present report indicates that wrongly timed and/or excessively dosed chronomodulated treatments could simultaneously produce undue toxicity and achieve poor efficacy, in good agreement with experimental data (Lévi et al., 2010). Thus, the opposite relationships between hematologic toxicity and efficacy outcomes according to chronomodulated or conventional drug delivery likely explain the observed similar overall survival of patients on either schedule (Giacchetti et al., 2006). Indeed, a specific feature of this trial was that chemotherapy doses were escalated

from one course to the next until reaching the maximum tolerated dose for each individual patient (Giacchetti et al., 2006). The occurrence of neutropenia resulted in comparable reductions in the actual dose intensities of 1-OHP and 5-FU in both treatment schedules (Figure 3, Table 1). However, drug doses were reduced also in case of severe clinical toxicities that were slightly more frequent in patients on chronoFLO4 (Table 1). No relationship was found between any efficacy endpoint and diarrhea on either delivery schedule, even though this symptom was the main dose-limiting toxicity on chronoFLO4 (Giacchetti et al., 2006) (data not shown). This finding suggests that bone marrow toxicity could induce a peculiar effect on the circadian timing system, possibly through the release of proinflammatory cytokines with circadian disruptive effects. Thus, elevated serum levels of transforming growth factor- α (TGF- α), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) were associated with circadian disruption and poor survival in patients with metastatic colorectal cancer (Rich et al., 2005).

Altogether, female patients were more likely than men to experience neutropenia (Table 1), in agreement with prior reports (Chansky et al., 2005; Diasio & Lu, 1994; Schwab et al., 2008). Each patient on chronoFLO4 received the same chronomodulated schedule with respective peak times of 1-OHP and 5-FU-LV delivery rates at 16:00 h and at 04:00 h, selected according to preclinical data from male mice, human tissue proliferation studies, and early clinical trials (Abrahamsen et al., 1997; Bjamason & Jordan, 2002; Bjamason et al., 1999, 2001; Buchi et al., 1991; Giacchetti, 2002; Giacchetti et al., 2006; Lévi et al., 2010; Smaaland et al., 1991, 1992a, 2002). A phase I–II clinical trial confirmed the better tolerability of the current chronoFLO4 in male patients, through exploring schedules with peak times of drug delivery staggered by ± 3 , ± 6 , ± 9 , or ± 12 h in separate cohorts of patients with metastatic colorectal cancer (Lévi et al., 2007b). In female patients, however, a large interpatient variability in optimal timing was apparent, with an ~ 6 -h phase advance of optimal circadian timing for the peak delivery rates of 1-OHP and 5-FU-LV (Lévi et al., 2007b).

CONCLUSION

In conclusion, we confirm here the principle that is currently advocated to guide non-circadian-based chemotherapy, i.e., lack of any neutropenia reflects insufficient drug dosing and, therefore, impaired efficacy. We show, however, that this principle does not apply to circadian-based delivery. Furthermore, severe neutropenia appears to be detrimental to the efficacy of chronomodulated chemotherapy. These results support the policy of dose escalation until maximum tolerated dose is reached in each patient for non-circadian-based chemotherapy (Frei & Canellos, 1980). Conversely, chronomodulated chemotherapy provides a novel treatment paradigm, where good tolerability is required for

achieving optimal efficacy (Bross et al., 1966). Cancer chronotherapeutics has thus entered a new era: the current main goal consists of the joint improvement of both the safety and efficacy of any chemotherapy regimen, through the adjustment of drug doses and circadian delivery patterns according to sex and other individual patient characteristics. Obviously, this novel strategy of chronopharmacological refinement and personalization requires prospective clinical validation.

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Fatigue et amaigrissement corporel, un groupe de symptômes à valeur pronostique sélective de la chronothérapie circadienne dans le cancer colorectal métastatique.

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Contexte: La chimiothérapie anticancéreuse vise à détruire sélectivement les cellules tumorales. Toutefois, la survenue d'une neutropénie, secondaire à la toxicité ostéomédullaire de la chimiothérapie, a été associée à une meilleure survie chez les patients recevant une chimiothérapie conventionnelle par 5-fluorouracile, leucovorine et oxaliplatine (FOLFOX2) pour cancer colorectal métastatique. Une tendance opposée a été retrouvée chez les patients traités par les mêmes agents administrés à des stades circadiens spécifiques, selon un schéma chronomodulé fixe (chronoFLO4). Nous formulons l'hypothèse que la disruption circadienne induite par la chimiothérapie détériore sélectivement l'efficacité de la chronothérapie, dont le fondement requière la fonctionnalité du système circadien.

Méthodes: La survie des patients présentant une fatigue et/ou un amaigrissement, groupe de symptômes associé à une disruption circadienne, a été comparée à la survie des patients sans aucune toxicité, ou présentant d'autres types de toxicité. Les données proviennent de l'essai clinique international randomisé de phase III comparant FOLFOX2 à chronoFLO4, aux doses maximales tolérées (EORTC 05963). Les patients ont été répartis en 4 catégories selon l'absence de toxicité, la survenue de 'fatigue-amaigrissement' seulement ou avec d'autres toxicités, ou la survenue d'autres toxicités seulement, pendant les premières deux cycles de chimiothérapie (n=543). Des modèles de Cox multivariés séparés ont été utilisés pour évaluer le rôle des catégories de toxicité sur le temps jusqu'à progression et sur la survie globale. **Résultats:** Le taux de patients avec 'fatigue-amaigrissement' seules ou avec d'autres toxicités était de 26.8% pour FOLFOX2 et de 25.5% pour chronoFLO4 (p=0.72). La survenue de quelque toxicité cliniquement significative était plus fréquente chez les femmes que chez les

hommes seulement sous chronoFLO4 ($p=0.003$). Aucune catégorie de toxicité n'était prédictive de temps jusqu'à progression ou de survie globale sous FOLFOX2. La survie médiane sous FOLFOX2 était de 17.1 mois [Limites de Confiance à 95%, 13.2 à 21.0] chez les patients avec 'fatigue-amaigrissement' et de 19.8 mois [17.9 à 21.7] dans les patients répartis dans les autres catégories toxiques ($p=0.12$). Inversement, les patients avec 'fatigue-amaigrissement' sous ChronoFLO4, présentaient un temps jusqu'à progression et une survie globale significativement plus courts, indépendamment des autres facteurs pronostiques ($p<0.0001$ and $p=0.001$, respectivement). Ainsi, la survie médiane était de 13.8 mois [10.4 à 17.2] pour les patients avec 'fatigue-amaigrissement' et de 21.1 mois [19.0 à 23.1] pour ceux sans ce groupe de symptômes. **Conclusion:** L'apparition précoce d'une fatigue et/ou d'un amaigrissement chimio-induits est survenue plus fréquemment chez les femmes que chez les hommes. Le groupe de symptômes 'fatigue-amaigrissement' était un facteur de mauvais pronostic, statistiquement significatif et indépendant, pour le temps jusqu'à progression et la survie globale, et ce, uniquement pour les patients sous chronothérapie. Le suivi dynamique de la fonction circadienne devrait permettre le diagnostic précoce de disruption circadienne afin d'optimiser rapidement le schéma d'administration chronomodulée, conduisant ainsi à une amélioration personnalisée de la tolérance et l'efficacité de la chronothérapie.

Fatigue and Weight-Loss Predict Survival With Circadian Chemotherapy For Metastatic Colorectal Cancer.

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RUNNING TITLE: Fatigue, weight loss and survival in metastatic colorectal cancer

CONFLICT OF INTEREST STATEMENT

All authors have no conflict of interest to declare.

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ABSTRACT

BACKGROUND: Cancer chemotherapy aims at a selective destruction of cancer cells. Neutropenia, an indication of chemotherapy-induced bone marrow lesions, is known to best predict survival in patients on a conventional schedule of 5-fluorouracil-leucovorin-oxaliplatin (FOLFOX2) for metastatic colorectal cancer, presumably as evidence of cell-killing efficacy. However, an opposite trend was found among patients receiving chronotherapy, in which the same drugs are delivered at specific circadian times, according to a fixed chronomodulated schedule (chronoflora). We hypothesized that drug-induced circadian disruption would selectively and adversely affect chronotherapy

efficacy. **METHODS:** The survival of patients with fatigue and weight loss (FWL), a symptom cluster associated with circadian disruption, was compared to that of patients with other clinical toxicities, based on data from an international phase III trial comparing FOLFOX2 with chronoFLO4, administered at near the maximum tolerated dose (EORTC 05963). Patients were categorized during the initial two courses of chemotherapy into 4 subgroups: 1) those with no clinical toxicity; 2) those with FWL alone; 3) those with FWL and other clinical toxicities; or 4) those with other clinical toxicities only, (study group; n=543). Separate multivariate Cox models were used to assess the role of toxicity categories on time to progression (TTP) and overall survival (OS).

RESULTS: The overall proportion of patients with FWL alone or that combined with other clinical toxicities was 26.8% for FOLFOX2 and 25.5% for chronoFLO4 (p=0.77). This toxicity was more frequent in females than in males only on chronoFLO4 (p=0.01), but not on FOLFOX2 (p=0.27). No clinical toxicity in patients on FOLFOX2 was associated with TTP or OS. Thus, median OS on FOLFOX2 was 17.1 months [95% CI, 13.2 to 21.0] in the patients with FWL as compared to 19.8 months [17.9 to 21.7] in those without this cluster (p = 0.17). Conversely, the patients with FWL on chronoFLO4, displayed significantly shorter TTP and OS, independent of other prognostic factors (p<0.0001 and p=0.001, respectively). The median survival of the patients with FWL was 13.8 months [10.4 to 17.2] as compared to 21.1 months [19.0 to 23.1] in those without this symptom cluster.

CONCLUSION: Early-onset chemotherapy-induced fatigue and/or weight loss was a significant and independent predictor of poor TTP and OS only among patients on chronotherapy. This symptom cluster occurred more frequently in female as compared to male patients. Dynamic monitoring of circadian function should detect early circadian disruption so as to readily optimize chronotherapy delivery and jointly improve safety and efficacy.

INTRODUCTION

The relationship between chemotherapy-induced toxicity and efficacy has been investigated in several disease settings. This has led to the hypothesis that the occurrence of specific side effects in an individual patient indicates adequacy of drug exposure thus justifying an adaptive dosing protocol (1-4). Moreover, results from clinical studies have repeatedly shown that the lack of drug-specific toxicities was associated with poor outcomes (5-25). As a result, toxic events have been considered surrogate pharmacodynamic markers, and used for adaptive dosing protocols (1-4). For instance, neutropenia has been reported as an independent predictor of prolonged survival in patients receiving chemotherapy for metastatic colorectal cancer (12, 26). We recently confirmed this finding in patients given a conventional schedule of 5-fluorouracil-leucovorin-oxaliplatin (FOLFOX2) in an international randomized trial, where chemotherapy doses were escalated until the maximum tolerated dose was reached in each patient (EORTC 05963). However, no positive relationship between neutropenia and survival was found in the patients receiving a circadian-based schedule involving the chronomodulated delivery of the same drugs at selected times of day or night (26). Experimental data indeed support careful and specific attention to optimal circadian dosing of anticancer drugs, with maximal antitumor efficacy usually resulting from chemotherapy administered near the circadian time corresponding to best tolerability (27, 28). Moreover, wrongly timed and/or highly dosed chemotherapy disrupts the circadian timing system that constitutes the foundation of chronotherapy. Circadian rhythms are generated within each cell by molecular clocks, consisting of interwoven transcription/translation feedback loops involving 15 clock genes (28, 29). The molecular clocks are, in turn, coordinated along the 24 h time scale by an array of physiological rhythms, which are generated by the suprachiasmatic nuclei (SCN), a circadian pacemaker in the hypothalamus. The SCN receives daily inputs from environmental cycles, and generates rhythmic physiological outputs, such as rest-activity, body temperature and hormonal secretions. The circadian timing system encompasses these molecular, cellular, physiologic and pacemaker components and generates 24-hour rhythms in anticancer drug metabolism and cellular proliferation (28-32). The circadian timing system of cancer patients has been assessed with continuous rest-activity monitoring using a wrist actigraph (33). Using this method, circadian disruption was detected in ~1/3 of cancer patients (33, 34). Baseline circadian disruption was robustly associated with fatigue and appetite loss in 251 patients with metastatic colorectal cancer (33-36). Fatigue and appetite loss were also found in subjects suffering from jet lag or who were engaged in shift work, two conditions that disrupt the circadian timing system (37, 38). Moreover, fatigue and body weight loss, an objective measure that reflects, among other factors, appetite loss, occurs more frequently in cancer patients with circadian rhythm alterations rather than in those with robust rhythms on chemotherapy (39). Weight loss was found to be associated with decreased physical activity in patients whose rest-activity pattern was monitored with wrist-actigraphy (40). Conversely the occurrence of neutropenia has not been found to be related to circadian disruption (33, 34). Here, we hypothesize that early-onset fatigue and/or weight loss reflect chemotherapy-induced circadian disruption, a condition which selectively interferes with the efficacy of circadian chemotherapy. To probe this, we perform a post-hoc analysis of data prospectively collected for an international, randomized, phase III trial (EORTC 05963), conducted in 564 chemo-naïve patients with metastatic colorectal cancer randomized to receive first-line chemotherapy with either a chronomodulated (ChronoFLO4) or a conventional (FOLFOX2) schedule, employing oxaliplatin (I-OHP), 5-fluorouracil (5-FU) and leucovorin (LV) (41).

PATIENTS AND METHODS

Aim of the study

The study examined the primary hypothesis that early onset fatigue and weight loss, occurring during the initial 4 weeks of treatment, selectively indicates a poor prognosis for the survival of patients receiving a fixed chronotherapy schedule for metastatic colorectal cancer. This symptom cluster was selected as being related to the occurrence of circadian disruption in cancer patients on chemotherapy (26). For this reason, we also assumed that this symptom cluster would not predict survival among patients receiving conventional chemotherapy. Secondary aims included the prognostic relevance of other main severe toxicities that have no known association with circadian disruption.

Study population and chemotherapy schedules

Patients with chemotherapy-naïve metastatic colorectal cancer were enrolled in EORTC 05963 trial between October 1998 and February 2002 (41). They were randomized to receive first line chemotherapy with 5-fluorouracil (5-FU) - leucovorin (LV) - oxaliplatin (I-OHP) either as a chronomodulated infusion (ChronoFLO4) or with a conventional, non time- stipulated schedule (FOLFOX2). ChronoFLO4 consisted in chronomodulated infusions of 5-FU-LV from 22h15 to 09h45 (with a peak flow rate at 04h00), alternating with I-OHP from 10h15 to 21h45 (with a peak flow rate at 16h00), over a duration of 4 days (41, 42). FOLFOX2 involved the administration of I-OHP and LV as concomitant 2-hour flat infusions on day 1, followed by 5-FU infusion at constant rate for 22 hours. On day 2, LV was also infused for 2 hours, and followed by constant rate 5-FU for another 22 hours (41, 43).

Courses were repeated every 14 days. The starting doses per course were 100 mg/m² for I-OHP, 1,200 mg/m² for LV, and 3,000 mg/m² for 5-FU. A 5-FU dose-escalation was planned, up to 3,600 mg/m² (41). Dose modifications and treatment delays followed the same guidelines in both arms (41).

Study population and toxicity evaluation

The landmark population of this study involves the patients receiving at least 2 courses of chemotherapy according to EORTC05963. This time span covers the initial 4 weeks on chemotherapy. Clinical and haematological toxicities were graded after each treatment course according to the National Cancer Institute Common Toxicity Criteria, version 2.0. Fatigue and appetite loss were rated by the medical oncologist in charge of the patient. Whilst chemotherapy-induced fatigue was reported after each course, appetite loss was not systematically assessed, but body weight was systematically measured before each chemotherapy course. Weight loss was chosen as a surrogate quantitative indicator of decreased appetite (44). We therefore considered the occurrence of a fatigue- weight loss cluster if the patient experienced either grade ≥ 2 fatigue or weight loss $\geq 5\%$ of baseline body weight over the initial two courses of chemotherapy. The clinical relevance of the cut-off values selected a priori for our study has been described elsewhere (18, 39, 44).

The following toxicities were not considered to be associated with circadian disruption: diarrhea, stomatitis, mucositis, hand-foot syndrome, peripheral sensory neuropathy, nausea, vomiting, leukopenia, neutropenia, anemia and thrombocytopenia. In order to

distinguish the respective impact of each type of toxicity, patients in each treatment group were categorized into four subgroups according to the occurrence of no toxicity (subgroup 1), fatigue- weight loss only (subgroup 2) or associated with other toxic events of grade ≥ 3 (subgroup 3), or other grade ≥ 3 toxicities only (subgroup 4).

Statistical methods

The primary endpoint of the current study was the association between toxicity and overall survival (OS), defined as the time between the end (day 14) of the second treatment course and the date of death irrespective of cause. Patients still alive at database locking (November 09th, 2007) or lost to follow-up were censored at the date of last information on vital status. At this date, after a median follow-up of 87 months (range: 68-108), 488 events had occurred (89.9%).

The proportions of patients in each toxicity subgroup were computed for each treatment modality. The rates were compared with a two-sided Chi square test. Actual dose intensities (per square meter per week) received in the first two courses of chemotherapy and throughout the whole treatment span were compared between the four patient categories with the non-parametric Mann-Whitney U test. The survival functions of the four patient subgroups defined by the occurrence of fatigue- weight loss and/or other toxicities following the initial two treatment courses were estimated with the Kaplan-Meier's method and compared with a Log rank test, separately in each treatment arm. The hazard ratio (HR) of an earlier death associated with the occurrence of fatigue- weight loss and/or other toxicities was estimated using Cox proportional hazards models, separately in each treatment arm. Multivariate prognostic models for OS included other parameters that were force-entered, whilst toxicity category was conditionally added, to explore whether it had an independent prognostic value above and beyond the other parameters considered. As a first step, an identification of the parameters predictive for the occurrence of any clinically relevant toxicity (fatigue- weight loss and/or other toxicities) was performed with a thorough screening of clinical features with a binary logistic univariate regression model. The characteristics included sex, age, baseline body mass index (BMI), performance status (PS) at inclusion (WHO scale), number of metastatic sites, percentage of liver involvement by tumor, dose intensities of 5-FU and I-OHP over the first two cycles, prior adjuvant chemotherapy, Dukes' stage at diagnosis, site of primary tumor, surgical resection of the primary tumor, prior surgery of metastases, baseline leukocyte counts and baseline alkaline phosphatase levels. Upon verification of the absence of collinearity ($rs[0.37]$), these parameters were added in a block in the prognostic model. Secondly, the subgroup category according to toxicity occurrence was added to the multivariate proportional hazard model. The same statistical procedure was performed to examine the independent predictive role of chemotherapy-induced fatigue- weight loss and/or other toxicities on time to progression (TTP), calculated from the end of the second course until documented disease progression, death, or last contact, whichever occurred first. A similar multivariate Cox hazard regression modelling was performed using separately the occurrence of fatigue- weight loss and the occurrence of other toxicities as covariates, to validate the specificity of either toxicity on TTP or OS, for each treatment modality. A multivariate binary logistic regression model was used to assess the predictive value for best objective tumor response (WHO criteria) of chemotherapy-induced toxicity. Further sensitivity analyses were performed excluding patients with missing data. Because for each endpoint two independent models were fitted for FOLFOX2 and for chronoFLO4 modalities respectively, the threshold for statistically significant differences was set at $p \leq 0.025$, according to a Bonferroni correction. All analyses were performed using PASW Statistics 18 software (SPSS Inc., USA).

Study population

Of 564 enrolled and randomized patients into EORTC 05963, 543 patients (96.3%) received at least two courses of chemotherapy and constituted the study population (Figure 1). The clinical and demographic features of the 272 patients on FOLFOX2 and the 271 patients on chronoFLO4 are presented according to the occurrence of fatigue-body weight loss and that of other toxicities (Supplemental Table 1). The study profile is recapitulated in Figure 1.

RESULTS

Toxicity rates according to treatment arm

Fatigue- weight loss was encountered in 73 patients (26.8%) on FOLFOX2, and 69 patients (25.5%) on chronoFLO4 ($p=0.72$), whereas other toxicities occurred in 40 patients (14.7%) and 54 patients (19.9%), respectively ($p=0.11$) (Figure 2). The co-occurrence of fatigue-weight loss and other toxicities was significantly more frequent on chronoFLO4 ($n=27$; 10.0%) than on FOLFOX2 ($n=14$; 5.1%) ($p=0.036$). Globally, the relative proportions of patients in each of the four toxicity categories did not differ according to treatment modality with statistical significance ($p=0.07$) (Figure 2). Females receiving chronoFLO4 were significantly more likely to develop toxicity than males on chronoFLO4 (Table 1). Patients with good baseline Performance Status (PS) displayed a significantly reduced risk of experiencing toxicity following either treatment schedule (Table 1).

Dose intensities

The actual dose intensities of 5-FU and I-OHP over the initial two treatment courses varied according to the toxicity category in the FOLFOX2 schedule ($p=0.008$ and $p=0.001$, respectively) but not in the chronoFLO4 one ($p=0.24$ and $p=0.20$, respectively) (Table 1).

Predictive value of toxicity on best objective tumor response

All outcomes (response rate, time to progression and overall survival) were similar independently of randomized treatment (41), but the range of outcomes according to the toxicity category was broader on chronoFLO4 (Table 2).

Objective response rates were similar in the four toxicity categories for patients on FOLFOX2 ($p=0.80$) (Table 2). Thus, neither fatigue-weight loss, nor other toxicities predicted an objective response on FOLFOX2 ($p \geq 0.33$). Conversely, patients experiencing both fatigue-

weight loss and other toxicities on chronoFLO4, displayed a 22.2% response rate, whereas in the other subgroups an objective response was observed in at least 40.5% of the patients (Table 2) ($p=0.06$).

Prognostic value of toxicity on time to progression

The four toxicity categories displayed similar curves for time to progression for patients on FOLFOX2 (log rank, $p=0.11$), without any apparent trend (Figure 3A). Conversely, on chronoFLO4, a significant difference in time to progression was found according to toxicity category (log rank $p < 0.0001$), with worse outcome in the patients with fatigue- weight loss with or without other toxicities (Figure 3B). This resulted in a higher risk of earlier progression on chronoFLO4 in patients with fatigue- weight loss, either alone ($p<0.0001$) or combined with other toxicities ($p=0.001$). No such relationship was found among patients with toxicities other than fatigue- weight loss ($p=0.59$). For patients on chronoFLO4, fatigue- weight loss, alone or combined with other toxicities, was confirmed as an independent prognostic indicator of the risk of an earlier progression, using multivariate Cox model ($p=0.002$ and $p=0.013$, respectively). No such relation was validated for in the patients with toxicities other than fatigue- weight loss ($p=0.54$) (Table 3). The multivariate models further confirmed the lack of predictive value of any toxicity category for TTP in the patients on FOLFOX2 (Table 3). In summary, the occurrence of fatigue- weight loss was unrelated to time to progression on FOLFOX2 ($p=0.07$) (Figure 3C), whilst it was strongly associated with a shorter TTP on chronoFLO4 ($p<0.0001$) (Figure 3D).

Prognostic value of toxicity on overall survival

Overall survival curves were similar in the four toxicity categories for the patients on FOLFOX2 (log rank $p=0.45$) (Figure 4A), with similar median survival durations ranging from 16.4 to 19.8 months (Table 2). However, the survival of patients on chronoFLO4 differed significantly as a function of the toxicity category (log rank $p<0.0001$), with medians ranging from 13.7 months (fatigue-weight loss category) to 21.6 months (no toxicity category) (Table 2; Figure 4B). Thus, the patients with fatigue- weight loss on chronoFLO4 displayed an increased risk of an earlier death (this cluster only, $p=0.002$; combined, $p<0.0001$) as compared to those without such toxicity. No relation was found on chronoFLO4 between survival and any other toxicities than fatigue- weight loss ($p=0.37$).

The increased risk of earlier death associated with fatigue-weight loss on chronoFLO4, either alone ($p=0.009$) or combined with other toxicities ($p=0.006$) remained significant, independent of the other known prognostic factors and parameters associated with these toxicities in a multivariate Cox's model (Table 3). The multivariate models confirmed the lack of prognostic value of toxicities other than fatigue-weight loss on chronoFLO4 and of any toxicity category for OS on FOLFOX2 (Table 3).

In aggregate, for patients on FOLFOX2, overall survival was not influenced by the occurrence of fatigue- weight loss ($p=0.12$) (Figure 4C) or any other categorized toxicity ($p=0.89$; not shown). Conversely, the occurrence of fatigue-weight loss (Figure 4D) ($p<0.0001$) or of other toxicities ($p=0.022$; not shown) on chronoFLO4 were each associated with a higher risk of earlier death. However, in the multivariate Cox model including other prognostic factors, only the occurrence of fatigue-weight loss remained a significant predictor of worse survival ($p=0.001$), whilst the occurrence of other toxicities did not in the final multivariate model ($p=0.08$, not shown).

The rate of missing data per item for the parameters here considered (blood counts, clinical toxicities and body weight), after the first two courses of chemotherapy, was of 1.3%. Sensitivity analyses performed eliminating patients with at least one missing data yielded strictly comparable results to those reported in the main landmark group (not shown).

DISCUSSION

The current study revealed that the advent of fatigue- weight loss during the initial 4 weeks of chemotherapy predicted reduced benefit from a fixed chronotherapy schedule in patients with metastatic colorectal cancer. No such relation was found with fatigue- weight loss in patients receiving non-time stipulated chemotherapy, or for patients experiencing other severe clinical or haematological toxicities. This is indeed the first clinical proof of a negative relation between toxicity and both time to progression and survival in oncology. Prior studies have invariably reported a positive association between defined toxicities and clinical outcome of patients with various malignancies receiving many kinds of anticancer agents (5-25). The specificity of this finding for chronotherapy supports a shared circadian biological mechanism correlating poor anticancer activity with poor tolerability (28). This study highlights fatigue - weight loss as a critical symptom cluster that impairs or indicates worse chronotherapy efficacy. Fatigue, anorexia and weight loss are frequent chemotherapy-induced complaints, which often cluster and may share common mechanisms, conceivably involving circadian disruption (36, 39, 45-53).

In the current study, toxicity was almost twice as frequent in females than in males on chronoFLO4. Thus the higher toxicity incidence in females could have been particularly detrimental for the efficacy of chronomodulated chemotherapy, whose biological bases require coordinated functional biological clocks, both in the central pacemaker and in the peripheral oscillators (28-30, 54-59). Indeed, we found a higher incidence of the concomitant occurrence of symptoms correlated with circadian disruption (fatigue and/or weight loss) together with other severe clinical and/or haematological toxicities, following treatment with chronoFLO4 (Figure 2). This finding supports the hypothesis that a non-optimal chronomodulated schedule, in terms of doses and/or administration times, could disrupt both the central pacemaker and the peripheral oscillators. This alteration at both levels would then result in respectively symptoms of circadian disruption (fatigue, weight loss) and in toxicity to peripheral tissues, such as the bone marrow (cytopenia) and the digestive mucosa (stomatitis, diarrhoea), which have been demonstrated to bear functional circadian clocks and/or circadian variations in cell cycle stages in humans (60-69).

Overall, frontline treatment with chronoFLO4 and FOLFOX2 yielded similar outcomes (41). Nonetheless, the range in survival differences according to the occurrence of early-onset toxicity was almost three times bigger on chronoFLO4 than on FOLFOX2 (Table 2). This finding supports the hypothesis that a fixed chronomodulated schedule can achieve concomitant survival improvement and good safety in a subgroup of patients as compared with non-time stipulated conventional delivery. However, when this fixed chronomodulated schedule is suboptimal for another subgroup of patients, this results in both shorter survival and higher toxicity. Instead, the variable administration times among patients and courses in the conventional schedule obscures these efficacy and safety differences related to the optimal delivery time (58).

Two main limitations of the current study should be acknowledged: firstly, patients were not randomized to develop toxicity. It could therefore be argued that other factors associated with fatigue- weight loss or other toxicities would be the main determinants of the survival differences observed. Toxic events would then merely form a surrogate marker of a poor prognosis related to other determinants. To rule out this possibility, we systematically proceeded with a thorough screening of all parameters possibly accounting for the induction of either toxicity (Table 1). All factors were forceably added to the Cox survival model, whereas the toxicity subgroup category was conditionally added to the model to test whether any of these events could be independently associated with survival. Indeed, the occurrence of chemotherapy-induced fatigue- weight loss remained an independent prognostic factor for both TTP and OS, despite the adjustment for the other parameters and other known prognostic factors in advanced colorectal cancer (70, 71) (Table 3). Secondly, clinical toxicity rating is a subjective parameter, whose estimation could vary among physicians. However, the subjectively-assessed performance status, which has been shown to display some degree of inter-observer variability among physicians (72, 73), still remains one of the main known prognostic factors in colorectal and other cancers (41, 70, 74, 75) and it is commonly used even as a stratification factor and/or inclusion criterion in many clinical trials. A subjective baseline patient-reported outcome can occasionally replace the subjective baseline physician-scaled performance status in the final prognostic models (76). Furthermore, a meta-analysis including individual patient data from 10,108 patients, reported that subjective, patient self-estimated pain, appetite loss and physical functioning at baseline predicted overall survival better than performance status (WHO scale) (77). Pertinent subjective parameters thus can add relevant prognostic information to sociodemographic and clinical measures. Circadian function can be non-invasively monitored with wrist-actigraphy, and objectively quantified in cancer patients (33, 34, 78, 79).

Alongside demonstrating the significant association between circadian disruption and subjective symptoms of mainly fatigue but also appetite loss in cancer patients (reviewed in (36)), continuous or repeated wrist-actigraphy monitoring has been proven to provide clinical information for the assessment of circadian disruption induced by general anaesthesia or by surgical procedures (80, 81). As a follow up to the finding in this study, our team is currently recording circadian rhythms before, during and after chemotherapy in order to precisely quantify the level of circadian disruption in patients on chemotherapy and to assess consequences for treatment tolerability and efficacy (82). The current study shows that the patients who display fatigue- weight loss within the initial 4 weeks of a fixed chronotherapy schedule are less likely to benefit from it. This provides a clinically relevant tool for further schedule optimization, which could benefit further from a quantitative approach, based on circadian rhythm monitoring. The early detection of circadian disruption could readily help its minimization through modifications of drug doses and/or administration time schedules resulting in the personalization of chronotherapy. Indeed, circadian-based schedules should achieve low toxicity in order to optimize efficacy, a paradigm which contrasts with that of conventional chemotherapy.

Figures and Tables legends

Figure 1.

Study flow-chart.

Figure 2.

Relative proportions of patients without toxicity (Group 1; white), with fatigue-weight loss only (Group 2; light gray), with both fatigue-weight loss and other toxicities (Group 3; grey stripes) and with other toxicities only (Group 4; dark grey), in each treatment arm.

Figure 3.

Kaplan-Meier curves depicting time to progression in each treatment arm (FOLFOX2, left panels and chronoFLO4, right panels). Top panels: categorization according to the four groups of patients defined by the occurrence of: no toxicity (Group 1; solid black lines), fatigue-weight loss only (Group 2; solid light gray lines), both fatigue-weight loss and other toxicities (Group 3; dashed light and dark grey lines) and other toxicities only (Group 4; solid dark gray lines). Bottom panels: categorization according to the occurrence (Groups 2 and 3; solid light gray lines) or not (Groups 1 and 4; dashed black lines) of fatigue-weight loss. P values derived from Log Rank test.

Figure 4.

Kaplan-Meier curves depicting overall survival in each treatment arm (FOLFOX2, left panels and chronoFLO4, right panels). Top panels: categorization according to the four groups of patients defined by the occurrence of: no toxicity (Group 1; solid black lines), fatigue-weight loss only (Group 2; solid light gray lines), both fatigue-weight loss and other toxicities (Group 3; dashed light and dark grey lines) and other toxicities only (Group 4; solid dark gray lines). Bottom panels: categorization according to the occurrence (Groups 2 and 3; solid light gray lines) or not (Groups 1 and 4; dashed black lines) of fatigue-weight loss. P values derived from Log Rank test.

Supplemental Table 1.

Clinical and demographical features of the four groups of patients defined by the occurrence or not of fatigue-weight loss and/or other toxicities following the initial two cycles of chemotherapy, in each randomized treatment arm. Group 1: no toxicity; group 2: fatigue-weight loss only; group 3: both fatigue-weight loss and other toxicities; group 4: other toxicities only. PS: performance status. WHO: World Health Organization. WBC: white blood cells. ALP: alkaline phosphatases. IU: international units. CEA: carcinoembryonic antigen. CA 19.9: Cancer-associated antigen 19.9. 5-FU: 5-fluorouracil. I-OHP: Oxaliplatin.

Table 1.

Univariate binary logistic regression for the identification of the parameters potentially predictive for the occurrence of any toxicity (fatigue-weight loss, other or both) in each treatment arm separately. The complete list of parameters accounted for is provided in the methods section. The parameters not significant in any model are not shown here. OR: odds ratio [95% Confidence Limits]. NS: not significant.

Table 2.

Clinical outcomes (objective response rate, time to progression, overall survival) according to the toxicity category separately in the two treatment arms.

Table 3.

Variables included in the multivariate Cox proportional hazard models for time to progression and overall survival in each treatment arm separately. HR: hazard ratio [95% Confidence Interval]. NS: not significant.

FIGURE 1.

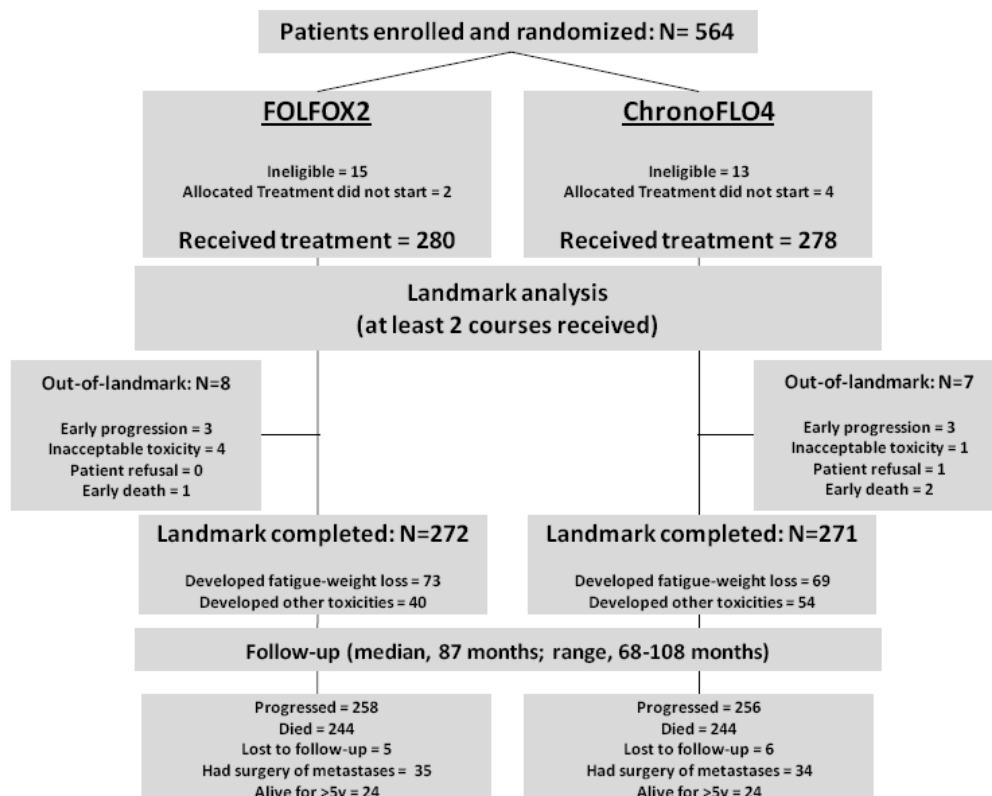


FIGURE 2.

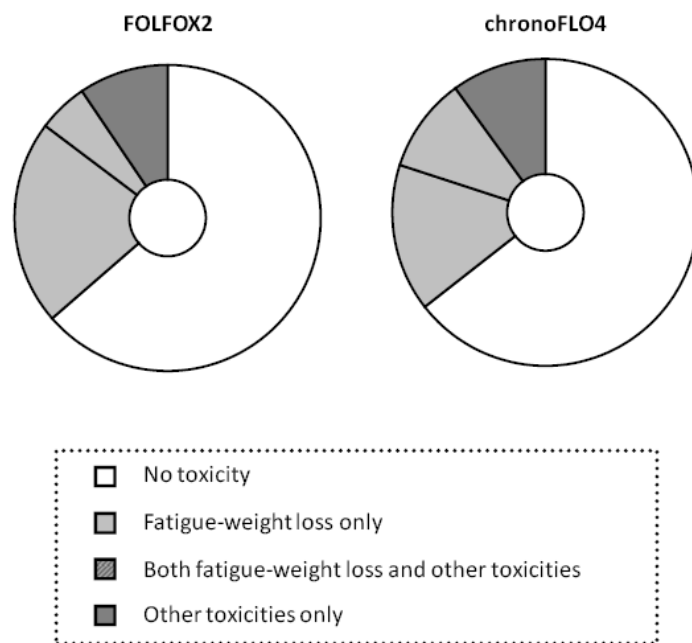


FIGURE 3.

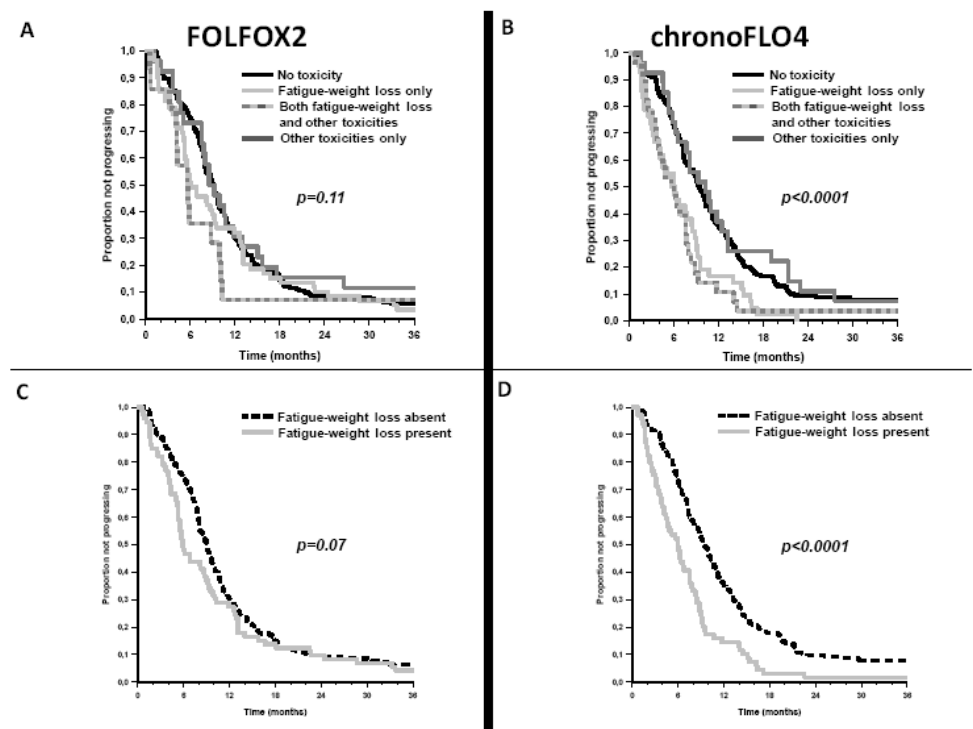
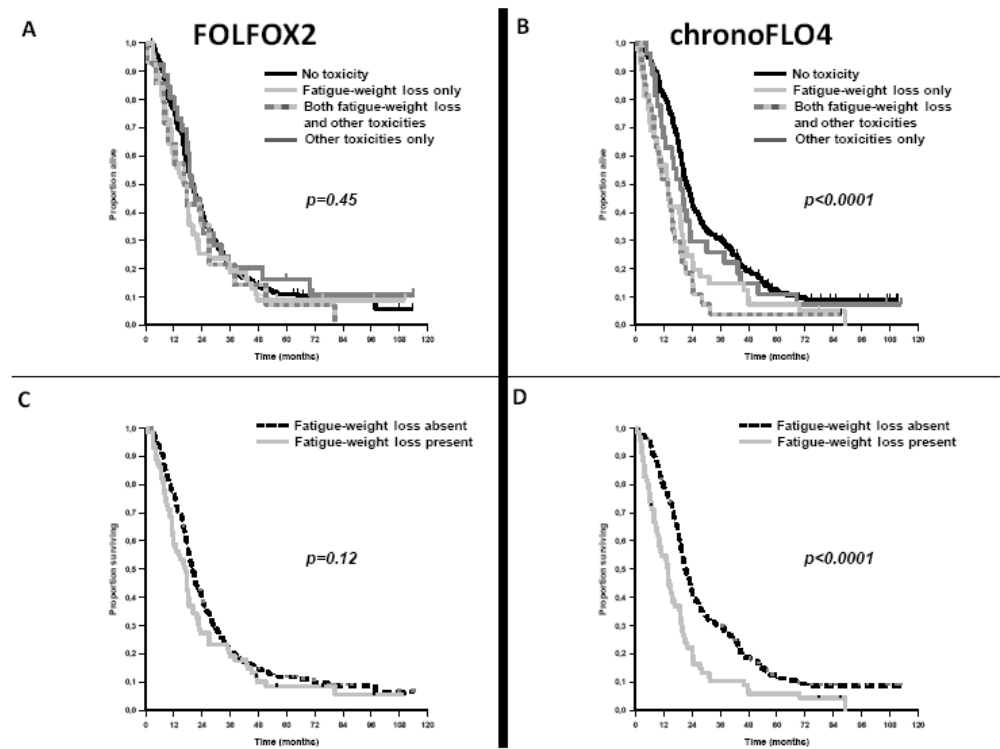


FIGURE 4.



SUPPLEMENTAL TABLE 1.

Characteristic		FOLFOX2					chronoFLO4				
		Group				All	Group				All
		1	2	3	4		1	2	3	4	
		(n=173)	(n=59)	(n=14)	(n=26)	(n=272)	(n=175)	(n=42)	(n=27)	(n=27)	(n=271)
Age (years)	Median (range)	62 (34-75)	63 (32-76)	63 (50-76)	63 (41-76)	62 (32-76)	61 (22-76)	59 (24-73)	68 (43-76)	62 (37-73)	62 (22-76)
Gender (%)	Female	36.4	47.5	35.7	46.2	39.7	33.1	52.4	55.6	48.1	39.9
	Male	63.6	52.5	64.3	53.8	60.3	66.9	47.6	44.4	51.9	60.1
PS at study entry (%)	0	54.3	30.5	35.7	73.1	50.0	52.6	28.6	29.6	66.7	48.0
	1	41.1	47.5	50.0	23.1	41.2	39.4	50.0	51.9	22.2	40.6
	2	4.6	22.0	14.3	3.8	8.8	8.0	21.4	18.5	11.1	11.4
Baseline Body Mass Index (Kg/m ²) (%)	Underweight (< 18.5)	4.6	1.7	0	8.0	4.1	4.0	2.4	7.4	7.4	4.4
	Normal (18.5-24.9)	44.5	59.3	71.4	36.0	48.3	44.6	71.4	44.4	44.4	48.7
	Overweight – Obese (≥ 25)	50.9	39.0	28.6	56.0	47.6	51.4	26.2	48.2	48.2	46.9
No. of metastatic sites (%)	1	43.4	25.4	50.0	57.7	41.2	41.7	47.6	51.9	40.7	43.5
	2	37.6	37.3	28.6	15.4	34.9	37.7	21.4	37.0	51.9	36.5
	≥3	19.1	37.3	21.4	26.9	23.9	20.6	31.0	11.1	7.4	19.9
Percentage of liver involvement by tumor (%)	None	17.3	8.5	28.6	23.1	16.5	12.0	16.7	25.9	11.1	14.0
	≤25%	48.6	49.1	50.0	42.3	48.2	48.0	33.3	33.3	66.7	46.1
	>25%	34.1	42.4	21.4	34.6	35.3	40.0	50.0	40.7	22.2	39.9
Prior adjuvant chemotherapy (%)	Yes	15.0	18.6	28.6	23.1	17.3	17.7	11.9	18.5	33.3	18.5
Synchronous metastases (%)	Yes	75.0	81.0	50.0	65.4	74.1	77.1	69.0	77.8	66.7	75.6
Site of primary tumor (%)	Colon	73.4	78.0	85.7	73.1	75.0	77.1	69.0	77.8	66.7	74.9
	Rectum	26.6	22.0	14.3	26.9	25.0	22.9	31.0	22.2	33.3	25.1
Prior surgery of primary tumor (%)	Yes	88.4	76.3	100	84.6	86.0	89.1	83.3	92.6	88.9	88.6
Prior surgery of metastases (%)	Yes	5.8	1.7	7.1	7.7	5.1	5.7	7.1	3.7	0	5.2
Baseline WBC (%)	≥ 10.0 x 10 ⁹ /L	21.4	32.2	14.3	15.4	22.8	19.5	21.4	33.3	7.4	20.0
Baseline ALP (%)	≥ 300 IU/L	23.7	37.3	28.6	19.2	26.5	25.1	42.9	40.7	25.9	29.5
Baseline CEA (%)	≥ 10 ng/mL	67.6	81.4	57.1	53.8	68.8	67.4	66.7	66.7	55.6	66.1

Baseline CA19.9 (%)	≥ 37 IU/L	47.4	55.9	50.0	34.6	48.2		48.0	42.9	37.0	40.7	45.4
5-FU dose intensity 8 (g/m ² /week)	Median	1.66	1.66	1.55	1.58	1.66		1.66	1.65	1.58	1.56	1.64
	Range	0.83- 1.88	1.18- 1.79	1.19- 1.72	0.95- 1.79	0.83- 1.88		0.90- 1.95	0.56- 1.73	0.91- 1.72	0.58- 1.72	0.56- 1.95
I-OHP dose intensity 8 (mg/m ² /week)	Median	51.9	51.9	50.9	51.7	51.9		51.9	51.9	51.4	51.1	51.9
	Range	0-58.0	41.2- 56.0	39.1- 53.9	32.6- 53.9	0-58.0		29.3- 61.5	18.7- 54.3	26.1- 53.9	11.7- 53.9	11.7- 61.5
PS (WHO) at day1 of 3 rd course (%)	0	50.9	33.9	7.1	46.2	44.5		43.4	4.8	11.1	29.6	32.8
	1	46.2	40.7	50.0	42.3	44.9		48.6	59.5	51.9	55.6	51.3
	≥2 or unknown	2.9	25.4	42.9	11.5	10.6		8.0	35.7	37.0	14.8	15.9
Total number of cycles	Median (range)	11 (2-49)	9 (3-18)	9 (2-16)	10 (2-22)	10 (2-49)		10 (2-38)	8 (2-34)	8 (2-23)	12 (4-44)	10 (2-44)
Global dose intensity of 5- FU 5 (g/m ² /week)	Median	1.43	1.46	1.18	1.28	1.40		1.41	1.38	1.21	1.13	1.38
	Range	0.87- 1.81	0.60- 1.82	0.90- 1.44	0.64- 1.75	0.60- 1.82		0.79- 1.95	0.69- 1.76	0.75- 1.72	0.61- 1.61	0.61- 1.95
Global dose intensity of I- OHP 5 (mg/m ² /week)	Median	39.5	43.0	36.6	37.5	39.9		39.8	41.3	34.9	34.3	39.2
	Range	9.4-55.7	19.1- 53.0	27.2- 48.4	14.4- 51.0	9.4-55.7		8.3-60.9	9.0-51.9	18.7- 53.9	4.4-50.8	4.4-60.9
Overall incidence of any G3-4 toxicity (%)	Yes	66.5	84.7	100	100	75.4		62.9	81.0	100	100	73.1

TABLE 1.

Variables	Values		Toxicity occurrence					
			FOLFOX2				chronoFLO4	
			OR	P			OR	p
Gender	Male		NS				1	0.003
	Female						2.19 [1.32-	
Baseline PS (WHO)	0		1	0.006	1		0.028	
	1		1.29 [0.76-		1.44 [0.84-			
	2		4.48 [1.78-		2.94 [1.32-			
Number of metastatic sites	1		1	0.05	NS			
	2		0.94 [0.52-					
	≥ 3	1.97 [1.05-						
Dose intensity	Quantitative	NS			0.96 [0.92-	0.038		
Dose intensity	Quantitative	0.11 [0.02-	0.007	0.15 [0.04-	0.004			

TABLE 2.

Outcome	FOLFOX2					chronoFLO4				
	Group				All	Group				All
	1	2	3	4		1	2	3	4	
	(n=173)	(n=59)	(n=14)	(n=26)	(n=272)	(n=175)	(n=42)	(n=27)	(n=27)	(n=271)
Objective Response Rate (%)	48.0	40.7	42.9	46.2	46.0	48.6	40.5	22.2	51.9	45.0
Median Time To Progression (months) [95% CI]	9.1 [8.0-10.2]	6.1 [3.4-8.9]	5.6 [3.0-8.2]	8.5 [6.1-10.9]	8.7 [7.9-9.4]	9.4 [8.5-10.4]	6.0 [3.9-8.2]	5.6 [3.1-8.2]	10.2 [5.8-14.6]	8.4 [7.4-9.3]
Median Overall Survival (months) [95% CI]	19.8 [17.7-22.0]	17.1 [12.2-22.0]	16.4 [7.2-25.6]	19.6 [15.7-23.4]	18.6 [17.0-20.1]	21.6 [19.2-24.0]	13.7 [8.8-18.6]	13.9 [8.6-19.2]	19.1 [12.6-25.7]	19.7 [18.4-21.0]

TABLE 3.

Parameter	Type/Values	Time to progression				Overall survival					
		FOLFOX2			chronoFLO4		FOLFOX2			chronoFLO4	
		HR	p		HR	p	HR	p		HR	p
Gender	Male	NS			1	0.012	1	0.001	1	0.013	
	Female				1.42 [1.08-1.87]		0.61 [0.46-0.82]		1.43 [1.08-1.89]		
Baseline PS (WHO)	0	1	<0.0001	NS		1	<0.0001	NS			
	1	1.49 [1.12-1.97]				1.51 [1.13-2.01]					
	2	2.82 [1.67-4.75]				4.43 [2.53-7.76]					
# of meta static sites	1	NS		NS		1	0.005	NS			
	2					1.17 [0.85-1.60]					
	≥ 3					1.81 [1.23-2.62]					
Baseline ALP (IU/L)	≤ 300	1	0.002	NS		1	<0.0001	NS			
	> 300	1.74 [1.25-2.49]				2.02 [1.44-2.83]					
	Unknown	1.67 [0.83-3.39]				1.31 [0.64-2.70]					
Toxicity group	1: no toxicity	NS		1	0.002	NS		1	0.006		
	2: fatigue-weight loss only			1.80 [1.23-2.67]				1.65 [1.13-2.39]			
	3: both fatigue-weight loss and other toxicities			1.82 [1.14-2.92]				1.92 [1.20-3.05]			
	4: other toxicities only			0.87 [0.55-1.37]				1.30 [0.82-2.07]			

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VI. DISCUSSION GENERALE

De nombreux aspects de la physiologie humaine présentent des oscillations périodiques sur 24 heures, permettant à l'Homme une meilleure adaptation aux variations cycliques de l'environnement externe (Panda, Hogenesch et al. 2002; Reppert and Weaver 2002; Hastings, Reddy et al. 2003; Gachon, Nagoshi et al. 2004; Lévi and Schibler 2007; Liu, Lewis et al. 2007; Reddy and O'Neill 2010). Ces rythmes biologiques endogènes sont coordonnés par le système circadien, et constituent une propriété fondamentale des êtres vivant (Panda, Hogenesch et al. 2002; Reppert and Weaver 2002; Hastings, Reddy et al. 2003; Gachon, Nagoshi et al. 2004; Lévi and Schibler 2007; Liu, Lewis et al. 2007; Reddy and O'Neill 2010) (cf chapitre II.2). Le système circadien est constitué d'un pacemaker central, les noyaux suprachiasmatiques, qui génèrent un ensemble de signaux rythmiques, lesquels coordonnent les horloges moléculaires qui résident dans chaque cellule de l'organisme. Ces horloges moléculaires font intervenir au moins quinze gènes connus, dit gènes de l'horloge (Innominato, Levi et al. 2010; Levi, Okyar et al. 2010). Une perturbation expérimentale du système circadien peut être provoquée notamment par la destruction physique des noyaux suprachiasmatiques, certaines modifications de l'environnement photopériodique, ou une mutation d'un ou plusieurs gènes de l'horloge circadienne (Okamura, Miyake et al. 1999; Zheng, Larkin et al. 1999; Filipski, King et al. 2002; Filipski, Delaunay et al. 2004; Filipski, King et al. 2004; Filipski, Innominato et al. 2005). Des altérations ou une disruption du système circadien ont été associées à plusieurs pathologies humaines, dont certains troubles du sommeil, certaines maladies psychiatriques, cardiovasculaires, métaboliques, endocriniennes, neuro-dégénératives, et aussi certains cancers (Penev, Kolker et al. 1998; Rajaratnam and Arendt 2001; Turek, Dugovic et al. 2001; Barger, Lockley et al. 2009; Scheer, Hilton et al. 2009; Arble, Ramsey et al. 2010; Reddy and O'Neill 2010; Etain, Milhiet et al. 2011; Harvey 2011; Huang, Ramsey et al. 2011). En particulier, la disruption circadienne a été associée à un risque significativement plus élevé de développement de cancers mammaire, prostatique, colorectal et endométrial chez l'homme (Davis, Mirick et al. 2001; Schernhammer, Laden et al. 2001; Schernhammer, Laden et al. 2003; Kubo, Ozasa et al. 2006; Conlon, Lightfoot et al. 2007; Viswanathan, Hankinson et al. 2007), et considérée comme une condition probablement carcinogène pour l'homme (Straif, Baan et al. 2007). En outre, la perturbation du système circadien observé chez les sujets intolérant au décalage horaire ou au travail posté a été associée à

l'apparition de plusieurs symptômes qui détériorent le bien-être du sujet (Waterhouse, Reilly et al. 1997; Drake, Roehrs et al. 2004; Foster and Wulff 2005; Reinberg, Ashkenazi et al. 2007; Waterhouse, Reilly et al. 2007) (cf chapitre II.4.2.2). De plus, la destruction anatomique du pacemaker circadien hypothalamique ou la suppression fonctionnelle du système circadien accélèrent la croissance tumorale (Filipski, King et al. 2002; Filipski, Delaunay et al. 2004; Filipski, Innominato et al. 2005; Filipski, Li et al. 2006; Filipski and Levi 2009) (cf chapitre II.4.1.1). Ces données expérimentales, auxquelles j'ai contribué avant d'entreprendre ma thèse, conduisent à considérer le système circadien comme un point de contrôle de la progression cancéreuse, dans les modèles expérimentaux. Dans mon travail de thèse, j'ai mis en évidence que la disruption circadienne, évaluée par l'actimétrie du poignet, était un facteur pronostic péjoratif de survie globale, indépendamment des facteurs pronostics connus (cf. articles #5 et #8). Ce résultat conforte les observations précédentes rapportées par notre Unité pour les patients atteints de cancer colorectal métastatique antérieurement traités (Mormont, Waterhouse et al. 2000), et par l'équipe de David Spiegel pour les patientes atteintes de cancer mammaire avancé (Sephton, Sapolsky et al. 2000). Mon travail identifie le paramètre I<O (index de dichotomie) comme le paramètre circadien qui possède la valeur pronostique la plus robuste, en comparaison du coefficient d'autocorrélation sur 24h (r_{24}), ou de l'amplitude circadienne. Au contraire, l'activité moyenne est dénuée de toute valeur pronostique. Cette étude internationale réalisée chez 130 patients propose un seuil de I<O de 97.5%, en deçà duquel la probabilité de survie diminue en proportion (cf. article #5).

Un travail en préparation auquel je contribue confirme cette valeur seuil de I<O à partir de l'analyse d'une base internationale de données d'actimétrie hébergée par notre Unité s'incluant 436 patients atteints de cancer colorectal métastatique. Les résultats montrent un doublement des médianes de survie sans progression et de survie globale chez les patients dont le rythme circadien persiste, en comparaison de ceux qui présentent une disruption circadienne. Dans chacune des études, les différences de survie selon le rythme circadien sont indépendante des facteurs pronostics connus, et persistent pendant plusieurs années après l'enregistrement. Ceci laisse penser que la disruption circadienne associée au cancer est une caractéristique fondamentale personnelle de l'interaction hôte-tumeur. Ces résultats justifient ainsi le développement de thérapies spécifiques visant à la restauration du système circadien, afin d'améliorer la survie des patients cancéreux sous traitement.

Le seuil quantitatif de I<O de 97.5% pourrait être utilisé comme objectif thérapeutique dans de telles études de resynchronisation des patients cancéreux. De plus, la méthodologie non invasive d'estimation du système circadien, fondée sur le rythme d'activité-repos, permet de réitérer les mesures de ce rythme chez les patients cancéreux, sans gêner leur vie courante.

Ainsi, j'ai mis en évidence, pour la première fois, qu'une disruption circadienne peut être provoquée par une chimiothérapie. Dans une étude portant sur 77 patients, j'ai montré que la disruption circadienne chimio-induite était aussi associée à une survie significativement plus courte, (cf. article #8). Cette observation confirme, dans d'autres conditions, l'importance du système circadien dans le contrôle de la maladie cancéreuse et met l'accent sur la nécessité d'éviter d'altérer ce système par la chimiothérapie. Dans leur ensemble, ces résultats justifient le développement d'une chronothérapie spécifique visant à la protection du système circadien contre une perturbation chimio-induite.

L'ensemble de ces résultats, obtenus dans des situations cliniques différentes, prouvent la pertinence de la chronobiologie en cancérologie, bien que les rythmes biologiques soient encore largement négligés dans la plupart des essais cliniques, des études translationnelles et des recherches fondamentales dans cette spécialité.

Les performances physiques et la prise alimentaire sont deux aspects de la physiologie humaine que contrôle le système circadien (Czeisler and Gooley 2007; Lévi and Schibler 2007). L'altération du système circadien observé chez les sujets intolérant au décalage horaire ou au travail posté, a été associée à une fatigue ou anorexie (Waterhouse, Reilly et al. 1997; Drake, Roehrs et al. 2004; Foster and Wulff 2005; Reinberg, Ashkenazi et al. 2007; Waterhouse, Reilly et al. 2007). Ces deux symptômes surviennent chez plus de 80% des patients cancéreux et sont provoqués par le cancer lui-même et par ses traitements médicaux et chirurgicaux (Fortner, Schwartzberg et al. 2007; Honea, Brant et al. 2007; Aprile, Ramoni et al. 2008; Aprile, Ramoni et al. 2009; Eng 2009; Kim, Jahan et al. 2009; Esper 2010; Molassiotis, Wengstrom et al. 2010; Xiao 2010). La survenue de ces symptômes contribue à détériorer la qualité de vie et le bien-être des patients. Aucun traitement actuel n'est véritablement efficace pour les combattre (Yavuzsen, Davis et al. 2005; Dy, Lorenz et al. 2008; Minton, Richardson et al. 2008). Il existe donc un réel besoin de thérapeutiques spécifiques de ce groupe de symptômes. J'ai mis en évidence une relation hautement statistiquement significative entre disruption circadienne et l'association fatigue-anorexie dans deux études réalisées chez un

total de 328 patients atteints de cancer colorectal métastatique (cf. articles #5, #6 et #8). La première étude concernait 251 patients où symptômes et rythme circadien étaient évalués avant le début d'une chimiothérapie. La seconde étude portait sur 77 patients en cours de chimiothérapie. Ces résultats fournissent une base physiopathologique pour guider le développement de traitements ciblant le système circadien, dans le but d'améliorer la fatigue et l'anorexie des patients atteints de cancer. En outre, j'ai démontré dans une étude portant sur 96 patients, que la perturbation du système circadien était associée à une moindre qualité de vie, dans sa globalité et dans différentes dimensions, notamment sociale (cf article °5). La vie sociale est aussi un synchroniseur important du système circadien de l'Homme (Hastings, Duffield et al. 1998; Stephan 2002; Honma, Hashimoto et al. 2003; Roenneberg, Daan et al. 2003; Mistlberger and Skene 2004; Mistlberger and Skene 2005; Roenneberg and Merrow 2007). L'altération du fonctionnement social est aussi associée à une survie globale plus courte, indépendamment des facteurs pronostiques connus, comme je l'ai montré dans une étude effectuée sur 443 patients atteints de cancer colorectal métastatique, dont la qualité de vie a été évaluée d'après leurs réponses au questionnaire EORTC QLQ-C30 (cf. article #4).

A ce jour, les mécanismes précis de la disruption circadienne induite par la tumeur et associée à la fatigue et à l'anorexie ne sont pas connus. J'ai précédemment formulé l'hypothèse qu'un des possibles mécanismes de la disruption circadienne liée au cancer fasse intervenir une action centrale des cytokines pro-inflammatoires (Rich, Innominato et al. 2005). En effet, une élévation des concentrations circulantes de TGF- α , de TNF- α , d'IL1 β , d'IL6 et de VEGF, a été associée à plusieurs symptômes systémiques tels que la fatigue, l'anorexie et la dépression en présence d'une infection aiguë, d'une néoplasie ou après administration de certaines de ces protéines dans le troisième ventricule cérébral (Coogan and Wyse 2008; Dantzer, O'Connor et al. 2008; Miller, Ancoli-Israel et al. 2008; Reyes-Gibby, Wu et al. 2008; Seruga, Zhang et al. 2008). Nous avons ainsi validé l'hypothèse d'une augmentation significative des concentrations sériques de TGF- α , de TNF- α et d'IL-6 chez les patients atteints de cancer colorectal métastatique non traités, dont le rythme circadien d'activité et de repos était altéré en comparaison de ceux dont le rythme était maintenu (Rich, Innominato et al. 2005).

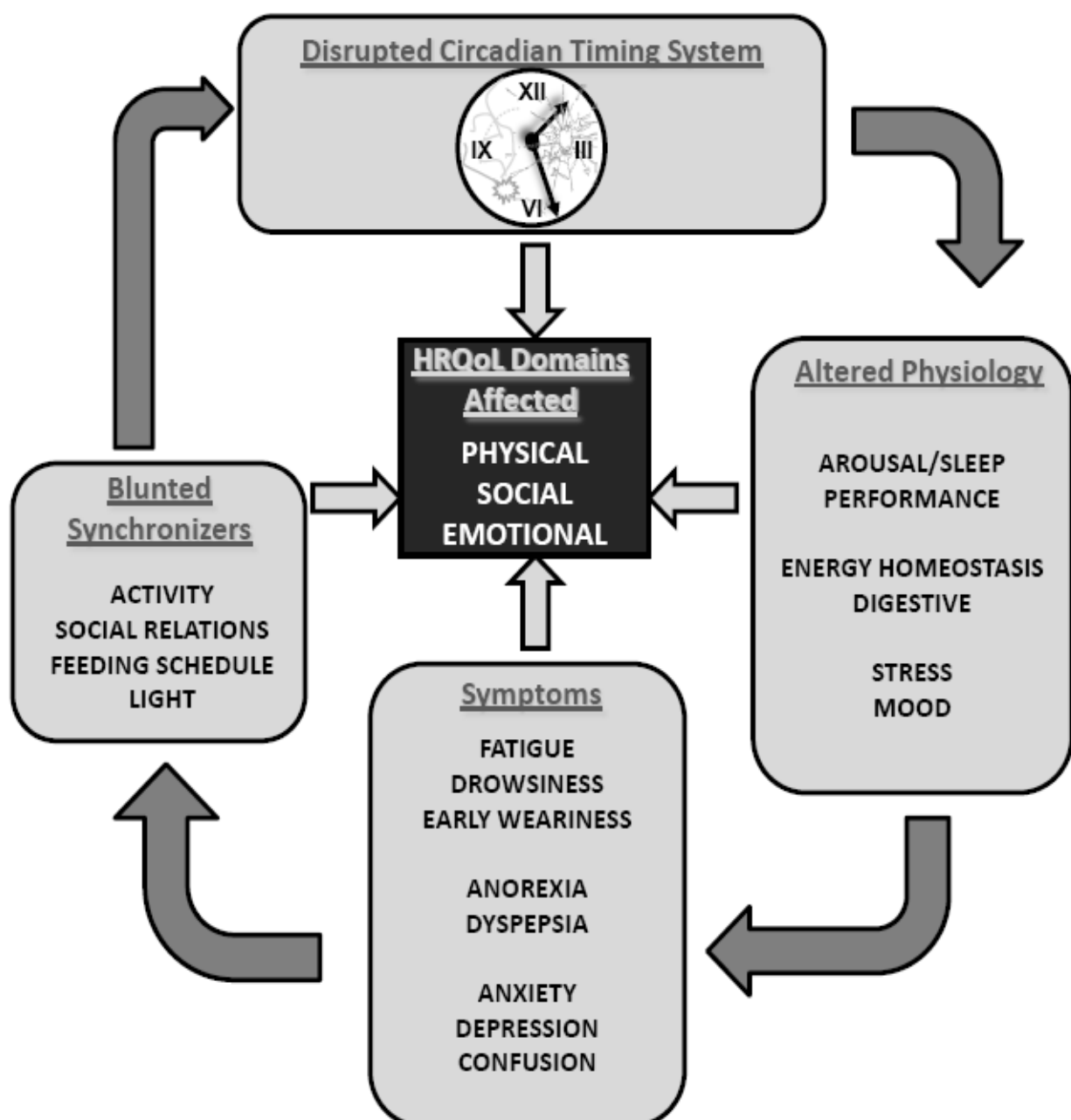
Actuellement, de nombreuses recherches ont pour but de bloquer l'effet des cytokines, principalement au niveau périphérique, afin d'augmenter la cytotoxicité

tumorale ou de diminuer la sarcopénie liée à la cachexie (Loberg, Bradley et al. 2007; MacDonald 2007; Meads, Hazlehurst et al. 2008; Tayal and Kalra 2008; Howlett, Menheniott et al. 2009; Weidle, Klostermann et al. 2010; Dodson, Baracos et al. 2011). Par contre, peu de recherches thérapeutiques concernent l'effet central des cytokines en cancérologie. Toutefois, plusieurs antagonistes ou anticorps ciblant les récepteurs de ces cytokines ou d'inhibiteurs de leurs voies de signalisation, sont disponibles ou en développement clinique avancé, ouvrant de nouvelles perspectives pour la thérapeutique de la disruption circadienne et des symptômes associés. Nous avons fourni une première preuve de principe de cette hypothèse thérapeutique lors d'une étude menée en collaboration avec S. Iacobelli et I. Iurisci (Université « G. d'Annunzio », Chieti, Italie) et T. Rich (University of Virginia, Charlottesville, USA) (Iurisci, Rich et al. 2007). Dans cette étude pilote, nous avons évalué l'effet du gefitinib, petite molécule inhibitrice du domaine tyrosine kinase de l'EGFR (ou HER-1), dont un ligand naturel est le TGF- α . L'effet du gefitinib, connu pour passer la membrane hémato-encéphalique (Ceresoli, Cappuzzo et al. 2004; Rich 2007; Papadatos-Pastos and Banerji 2011), a été étudié sur le rythme circadien d'activité et repos, les symptômes et la qualité de vie chez 10 patients atteints de cancer bronchique avancé. L'administration matinale de gefitinib a amélioré la fonction circadienne chez 75% des patients qui présentaient une disruption circadienne documentée par actimétrie. La restauration du rythme circadien par le gefitinib s'accompagnait d'une amélioration cliniquement significative de la qualité de vie, des performances physiques, et d'une réduction de la fatigue et d'anorexie, sans aucun effet antitumoral direct évident (Iurisci, Rich et al. 2007).

La perturbation du système circadien par le travail posté ou le décalage horaire, provoque la survenue de symptômes associés à ces conditions. Notamment la fatigue et l'anorexie sont la conséquence de l'altération du contrôle circadien de la performance physique et de la prise alimentaire. Cependant, la fatigue, l'anorexie et l'altération du fonctionnement social, entre autres, peuvent à leur tour contribuer à l'émoussement des trois synchroniseurs critiques chez l'Homme, l'activité physique, l'activité physique,

Figure 22. Hypothèse de travail sur les relations bidirectionnelles entre symptômes et perturbation du système circadien chez l'Homme.

Une disruption du système circadien induite par le cancer s'associe à des altérations de la physiologiques à plusieurs niveaux. Ces altérations provoquent des symptômes qui rendent moins régulier l'effet des synchroniseurs externes, entrainant un cercle vicieux qui perturbe davantage le système circadien.



la prise alimentaire et la vie sociale (Hastings, Duffield et al. 1998; Stephan 2002; Honma, Hashimoto et al. 2003; Roenneberg, Daan et al. 2003; Mistlberger and Skene 2004; Mistlberger and Skene 2005; Roenneberg and Merrow 2007). Ainsi, la corrélation entre disruption circadienne et symptômes généraux est probablement bidirectionnelle (Figure 27). Il en résulterait un cercle vicieux d'où les patients ne peuvent sortir sans thérapeutique spécifique efficace, d'où son réel besoin.

La fatigue et l'anorexie sont aussi deux symptômes toxiques induits par la chimiothérapie (Aprile, Ramoni et al. 2008; Aprile, Ramoni et al. 2009; Eng 2009). Leurs mécanismes sont mal connus, mais l'augmentation des concentrations sanguines des cytokines pro-inflammatoires a été constatée sous chimiothérapie (Geinitz, Zimmermann et al. 2001; Bower, Ganz et al. 2002; Cleeland, Bennett et al. 2003; Mills, Parker et al. 2004; Puztai, Mendoza et al. 2004; Mills, Parker et al. 2005; Collado-Hidalgo, Bower et al. 2006; Wood, Nail et al. 2006; Coogan and Wyse 2008; Jager, Sleijfer et al. 2008; Miller, Ancoli-Israel et al. 2008; Mills, Ancoli-Israel et al. 2008; Myers 2008). Cette élévation a été retrouvée dans les jours suivant le début du traitement, en concomitance avec la survenue des symptômes tels que la fatigue et l'anorexie. Cette coïncidence permet d'envisager le rôle des cytokines dans la toxicité centrale de la chimiothérapie (Myers 2008). De plus, une perturbation du rythme d'activité-repos a été rapportée pendant l'administration de chimiothérapie chez l'Homme (Berger and Higginbotham 2000; Roscoe, Morrow et al. 2002; Fouladiun, Korner et al. 2007; Iurisci, Rich et al. 2007; Savard, Liu et al. 2009; Berger, Grem et al. 2010; Innominato, Levi et al. 2010; Levi, Okyar et al. 2010). Cependant peu d'études longitudinales permettent d'appréhender la dynamique du système circadien des patients sous chimiothérapie et son impact clinique.

Chez 77 patients étudiés par actimétrie pendant chimiothérapie, je n'ai retrouvé aucune association entre toxicités cliniques et/ou hématologiques sévères et disruption circadienne (cf. article #8). Par contre, j'ai observé une incidence plus élevée d'amaigrissement et de fatigue, cliniquement pertinents, chez les patients présentant une altération chimio-induite du rythme circadien d'activité et de repos (cf. article #8). L'amaigrissement peut être la conséquence de la survenue d'autres toxicités - et notamment, nausées, vomissements, diarrhée, et/ou l'anorexie. De ces toxicités, seule l'anorexie a été associée à une perturbation du rythme circadien d'activité-repos (Mormont, Waterhouse et al. 2000; Mormont and Waterhouse 2002) (cf. articles #5 et #6). L'amaigrissement lui-même a été associé à une altération de l'activité physique des patients cancéreux (Fouladiun, Korner et al. 2007). Ces

résultats me conduisent à formuler l'hypothèse que la perte de poids résulte principalement de l'anorexie et que, sa combinaison avec la fatigue constitue un groupe de symptômes associé à une disruption circadienne.

J'ai ainsi mis en évidence qu'une chimiothérapie peut modifier les rythmes circadiens du patient parfois de façon importante et durable (cf. article #7). Cette démonstration clinique complète les données expérimentales de notre Unité de Recherche et d'autres équipes (Ohdo, Koyanagi et al. 2001; Li, Kanekal et al. 2006; Li and Levi 2007; Ahowesso, Li et al. 2011), et valide, en recherche translationnelle, les hypothèses issues de la recherche fondamentales (cf. chapitre II.5.4). En effet, chez la Souris, l'administration de 12 médicaments anticancéreux de diverses classes pharmacologiques, provoque une disruption impliquant plusieurs niveaux d'organisation du système circadien: désorganisation des rythmes physiologiques de la température corporelle, de l'activité locomotrice, et des sécrétions glucocorticoïdes, mais aussi répression des gènes de l'horloge moléculaire dans le pacemaker central et les cellules du foie, de la surrénale, etc. (Ohdo, Wang et al. 2000; Ohdo, Koyanagi et al. 2001; Li, Kanekal et al. 2006; Li and Levi 2007; Ahowesso, Li et al. 2011). L'importance des altérations circadiennes chimio-induites dépend de la dose et de l'horaire d'administration. En particulier, la disruption circadienne est moindre après administration du cytotoxique au stade circadien de meilleure tolérance (Ohdo, Koyanagi et al. 2001; Li, Kanekal et al. 2006; Li and Levi 2007; Ahowesso, Li et al. 2011). L'hypothèse mécanistique, déjà formulée, présume qu'une augmentation des cytokines circulantes après chimiothérapie peut altérer le fonctionnement du pacemaker hypothalamique et provoquer ainsi une disruption de la physiologie circadienne. D'autres données révèlent un rôle de l'horloge circadienne moléculaire dans la pathogenèse de la disruption circadienne chimio-induite. En effet, plusieurs agents anticancéreux modifient les rythmes des gènes de l'horloge moléculaire *in vitro* et *in vivo* (Ohdo, Koyanagi et al. 2001; Terazono, Hamdan et al. 2008; Levi, Okyar et al. 2010) (cf chapitre II.5.4). De plus, le cycle cellulaire et l'horloge circadienne sont interconnectés par plusieurs mécanismes moléculaires, qui font intervenir le contrôle de Wee1 à la transition G₂/M et le contrôle de P21 à la transition G₁/S par le complexe CLOCK::BMAL1 (Matsuo, Yamaguchi et al. 2003; Schibler 2003; Hunt and Sassone-Corsi 2007; Levi, Filipinski et al. 2007; Grechez-Cassiau, Rayet et al. 2008; Okyar and Lévi 2008). En outre, plusieurs protéines du cycle cellulaire telles que Chk1 et Chk2 pourraient à leur tour réguler l'horloge circadienne moléculaire (Unsal-Kacmaz, Mullen et al. 2005; Iurisci, Filipinski et al. 2006; Kondratov and Antoch 2007; Yoshizawa-Sugata and

Masai 2007; Kemp, Akan et al. 2010; Yang, Guo et al. 2010; Yang, Wood et al. 2010; Cotta-Ramusino, McDonald et al. 2011). Les lésions de l'ADN, induites par les radiations ionisantes ou certains xénobiotiques peuvent déphaser l'horloge moléculaire circadienne, mais aussi le pacemaker hypothalamique (Oklejewicz, Destici et al. 2008). La combinaison d'altérations aux niveaux du pacemaker circadien cérébral et des horloges moléculaires périphériques pourrait provoquer l'effet macroscopique de disruption circadienne sous chimiothérapie. De plus, les agents cytotoxiques eux-mêmes sont souvent associés à des médicaments de supports - antiémétiques, facteurs de croissance hématopoïétiques, glucocorticoïdes, etc.

Plusieurs médicaments de support peuvent modifier le système circadien (cf chapitre II.5.4.1). Par exemple, les glucocorticoïdes représentent un des plus puissants synchroniseurs des horloges moléculaires périphériques *in vitro* et *in vivo* (Balsalobre, Brown et al. 2000; Kiessling, Eichele et al. 2010). L'un des mécanismes de la synchronisation des horloges moléculaires par les glucocorticoïdes implique leur effet direct sur la transcription du gène *Per2* (So, Bernal et al. 2009; Dibner, Schibler et al. 2010). L'administration de doses pharmacologiques très élevées et l'utilisation de formulations à libération parfois très prolongée entraîne une exposition aux glucocorticoïdes, sans rapport avec le rythme circadien physiologique de la cortisolémie (cf chapitre II.3.4). Leurs conséquences néfastes pour la Santé ont été montrées dans d'autres pathologies, en particulier respiratoires, et rhumatologiques, où le respect du rythme circadien du cortisol minimise les complications de la corticothérapie.

Des médicaments antiémétiques de différentes classes sont souvent combinés pour prévenir ou traiter les nausées et vomissements chimio-induits. Les noyaux suprachiasmatiques sont équipés de récepteurs de la plupart des neurotransmetteurs, que bloquent ces antiémétiques (cf. chapitre II.5.4.1) (Figure 25). Ainsi, les agents anti-sérotoninergiques, anti-neurokinin-1 (NK1) tel que l'aprepitant ou pro-GABAergiques, tels que les benzodiazepines, pourraient modifier le pacemaker central et ses 'sorties' physiologiques. L'ondansetron, inhibiteur sélectif du récepteur de type 3 de la sérotonine (5-HT₃) modifie le rythme de la température corporelle chez la Souris selon le stade circadien d'administration (Khedhaier, Ben Attia et al. 2003).

L'ensemble de ces résultats nous incitent à examiner les propriétés chronobiotiques des agents anticancéreux. En effet, ces agents peuvent modifier profondément le

système circadien. La disruption circadienne serait ainsi un nouveau type de toxicité de la chimiothérapie ignoré jusqu'ici.

La dynamique complexe du système circadien d'un patient sous chimiothérapie, nécessite d'en préciser les mécanismes afin de mieux cibler et ajuster les interventions chronothérapeutiques. Il était donc nécessaire de développer des méthodes peu invasives de monitoring continu du système circadien. L'actimétrie du poignet a permis d'évaluer la fonction circadienne centrale avant, pendant et après chimiothérapie. Une fois montrées la pertinence clinique du suivi du système circadien des patients sous traitements, et les limites de l'actimétrie, il est apparu nécessaire de combiner cette méthode à l'enregistrement du rythme thermique (cf. article #7).

En effet, la température corporelle centrale, dont le profil présente une allure inversée par rapport à la température superficielle, est actuellement considérée comme le rythme circadien le plus précis pour l'estimation de la phase interne de l'individu (Klerman, Gershengorn et al. 2002; Klerman 2005; Koukkari and Sothorn 2006). Il représente aussi un effecteur de la synchronisation des horloges périphériques, via ses effets sur les protéines thermosensibles - HSP et CIRBP (Brown, Zimbrunn et al. 2002; Buhr, Yoo et al. 2010; Li, Delaunay et al. 2010). Cependant, la mesure continue du rythme thermique central fait appel à une méthodologie complexe et invasive, difficilement acceptable par des patients cancéreux sous traitement (cf. chapitre II.3.2). Pour pallier à cette limitation, nous avons procédé en deux directions. D'une part, nous avons mesuré la température cutanée superficielle, à l'aide de patches cutanés équipés de micro enregistreurs thermiques-émetteurs, en coopération avec A. Gorbach (NIBIB, NIH, Bethesda, USA) (cf. article #7). D'autre part, j'ai participé au développement et à la mise au point d'un système innovant de mesure de la température corporelle centrale, avec Francis Lévi et Jacques Beau, en coopération avec la Société BBRAUN Medical (Celsite ® Rhythm™). Il s'agit d'un thermomètre inclus dans un site d'accès

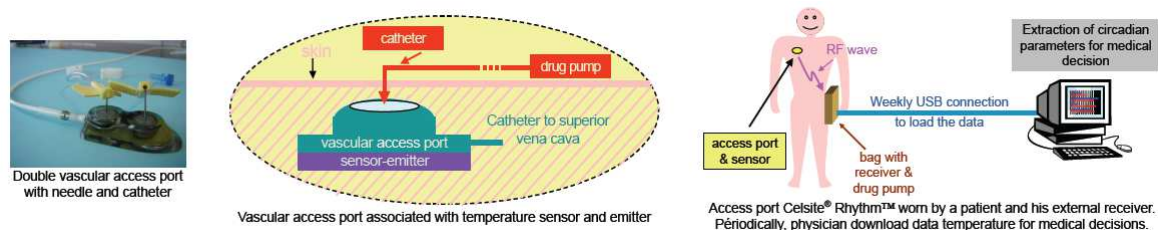


Figure 23. Principe de développement du Celsite Rhythm™ (panneaux central et à droite) et réalisation du prototype (photo de gauche)

vasculaire central implanté, destiné à l'administration de chimiothérapie. Ce système mesure la température centrale toutes les dix minutes et transmet les données à un récepteur extérieur, avec une autonomie de 2 ans (Figure 28).

Le Celsite Rhythm permettra d'obtenir cents-quarante-quatre mesures de température corporelle sous-cutanée profonde par 24 heures, pendant au moins deux ans, avec une gêne minime pour le patient (récepteur externe). Cette information, sera essentielle pour l'ajustement précis de la chimiothérapie chronomodulée selon la phase interne du pacemaker circadien de chaque patient, renseignée par le rythme thermique. L'enregistrement thermique pourra être combiné à l'actimétrie, afin de suivre concomitamment deux biomarqueurs indépendants du système circadien des patients sous chimiothérapie. La pertinence préclinique de ces deux rythmes pour la chronothérapeutique expérimentale des cancers a été démontrée chez la Souris (Levi, Okyar et al. 2010) , de même que leur importance clinique (cf. articles #5, #6, #8 et #11). Le monitoring continu des rythmes d'activité-repos et de la température corporelle aidera à définir la fréquence des mesures d'autres biomarqueurs circadiens potentiels, tels que les rythmes transcriptionnels des gènes de l'horloge moléculaire ou d'autres gènes d'intérêt, que nous mettons au point actuellement dans notre Unité. La combinaison de plusieurs biomarqueurs circadiens, mesurés de façon non ou peu invasive, chez le même patient conduira à mieux comprendre les effets chronobiotiques des agents anticancéreux, et à en modéliser les actions au plan mathématique, afin de proposer et valider de nouvelles approches chronothérapeutiques pour prévenir ou traiter la disruption circadienne chimioinduite. D'autres rythmes bio-marqueurs de la fonction du système circadien existent, notamment celui du cortisol circulant (Klerman, Gershengorn et al. 2002; Mormont, Langouet et al. 2002; Klerman 2005). Ce rythme est le résultat du contrôle temporel de l'horloge centrale, mais aussi de l'horloge moléculaire endogène des glandes surrénaliennes endocrines (Chung, Son et al. 2011) (cf. chapitre II.3.4). Le rythme circadien du cortisol, comme celui de la température centrale, est aussi un effecteur de la coordination des horloges moléculaires périphériques (Balsalobre, Brown et al. 2000; Kiessling, Eichele et al. 2010). Le rythme du cortisol salivaire, reflet du rythme de la cortisolémie libre, est aussi un facteur pronostique indépendant de survie chez les patientes atteintes de cancer du sein métastatique, comme démontré par David Spiegel et ses collaborateurs (Sephton, Sapolsky et al. 2000). Par contre, une telle relation avec la survie globale n'a pas été retrouvée chez les patients atteints de cancer colorectal métastatique dans notre Unité (Mormon, Bogdan et al. 2002). Il s'agit bien de deux

biomarqueurs indépendants du système circadien, puisque je n'ai retrouvé aucune corrélation entre rythme circadien du cortisol sérique ou salivaire et rythme circadien d'activité-repos (cf. article #6). Cette constatation nous incite à combiner plusieurs biomarqueurs circadiens pour mieux appréhender divers composants du système circadien.

L'intérêt de cette approche est souligné par la coïncidence entre chronotolérance et chronoefficacité, à la base d'une optimisation conjointe de la tolérance et de l'efficacité de la chronothérapie. La chimiothérapie conventionnelle s'appuie sur le concept de traitement aux doses maximales tolérées. A cet égard, les résultats que j'ai obtenus avec le schéma conventionnel FOLFOX confirment la valeur pronostique de la neutropénie pour la survie, en accord avec plusieurs études antérieures (Cameron, Massie et al. 2003; Di Maio, Gridelli et al. 2005; Yamanaka, Matsumoto et al. 2007; Kishida, Kawahara et al. 2009; Shitara, Matsuo et al. 2009; Kim, Park et al. 2010; Shitara, Matsuo et al. 2010; Shitara, Matsuo et al. 2010; Lee, Gurney et al. 2011) (cf. articles #10 et #11). A l'inverse, pour la chronothérapie par chronoFLO la survie est prolongée en l'absence de toxicité hématologique ou clinique sévère (cf. articles #10 et #11). Ainsi le principe de chronothérapie repose-t-il sur l'association positive entre efficacité et tolérance. Ces résultats suggèrent qu'une prévention de l'altération circadienne chimio-induite pourrait être bénéfique tant pour la tolérance que l'efficacité des traitements anticancéreux.

Les données cliniques de la chronothérapeutique des cancers sont résumées dans une revue récente que j'ai compilée pendant mon travail de thèse (cf article °2). En outre, j'ai participé à l'application dans la routine clinique et l'évaluation des résultats de deux schémas de chimiothérapie chronomodulée contre le cancer colorectal métastatique, en parallèle à ce travail de thèse de science (cf. articles annexes #12 et #13).

L'optimisation de la chimiothérapie chronomodulée permettrait de préserver le système circadien d'une altération chimio-induite, et, par conséquence, d'améliorer conjointement la qualité de vie et la survie des patients atteints d'un cancer. En concomitance, l'absence de limitation à la performance physique, à la vie sociale et à l'appétit chez les patients cancéreux permettrait de ne pas souffrir de fatigue et d'anorexie et de ne gêner pas à leur qualité de vie, préservant ainsi l'efficacité de trois synchroniseurs du système circadien, l'activité physique, la prise alimentaire et la vie sociale. Par conséquent, le système circadien jouerait de contrôle de la croissance tumorale, favorisant une moindre évolutivité de la maladie néoplasique.

Ce travail de thèse a ainsi fourni de nouvelles preuves de l'importance et de la pertinence clinique de la disruption circadienne en cancérologie, et a démontré la faisabilité et l'intérêt d'une évaluation multifactorielle et dynamique de la fonction circadienne en cours de chimiothérapie. Ces informations sont ainsi fondamentales pour la mise en œuvre d'une nouvelle approche chronothérapeutique, mais diverses questions restent encore à résoudre. En particulier, les mécanismes précis de la disruption circadienne chimio-induite et les effets précis des médicaments anticancéreux sur plusieurs rythmes physiologiques et moléculaires du système circadien.

VII. CONCLUSIONS ET PERSPECTIVES

Les travaux présentés concernent l'étude du système circadien des patients atteints de cancer colorectal métastatique et de son rôle dans la progression tumorale, la qualité de vie et la survie. L'objectif est d'identifier l'intérêt potentiel d'une approche thérapeutique ciblant le système circadien en oncologie. Les 11 articles présentés qui constituent les matériaux de ma thèse s'inscrivent dans cette perspective, en apportant les éléments de réponse suivants:

- a) la démonstration qu'une perturbation du rythme circadien d'activité et de repos, mise en évidence avant le début d'une chimiothérapie, constitue un facteur pronostic péjoratif de survie chez les patients cancéreux, indépendamment des autres paramètres cliniques et biologiques. J'ai validé cette conclusion dans une étude internationale, chez des patients qui n'avaient préalablement reçu aucune chimiothérapie, et quantifié le seuil critique de $I < 0$, le paramètre du rythme que j'ai identifié comme le plus cliniquement pertinent.
- b) la confirmation qu'une perturbation du rythme circadien d'activité et de repos est associée à une moindre qualité de vie globale, et à une diminution des dimensions physique, sociale et de rôle de la qualité de vie, chez les patients cancéreux;
- c) la confirmation que la dimension sociale de la qualité de vie, estimée par le patient, est un facteur pronostique indépendant de survie;
- d) la mise en évidence d'une association négative robuste entre rythme circadien d'activité et de repos et fatigue et anorexie;
- e) la démonstration que l'administration de chimiothérapie peut altérer, de façon parfois considérable et durable, le fonctionnement du système circadien des patients;
- f) la démonstration que l'enregistrement continu de l'activité locomotrice et de la température cutanée superficielle, deux biomarqueurs du système circadien, est réalisable de façon peu invasive et ambulatoire chez les patients cancéreux sous chimiothérapie. Ceci a permis la première démonstration que la perturbation du rythme circadien d'activité et de repos induite par la chimiothérapie était aussi un facteur pronostique indépendant de survie ;
- g) la première démonstration que la toxicité provoquée par la chimiothérapie chronomodulée est associée à une réduction de son efficacité. Ce résultat me

conduit à proposer l'hypothèse que la perturbation chimio-induite du système circadien, associée aux toxicités systémiques, en est la raison principale.

h) l'établissement du rôle critique du sexe des patients dans l'optimisation chronothérapeutique. J'ai mis en évidence que la chronothérapie provoquait une moindre toxicité et une efficacité supérieure en comparaison d'un schéma conventionnel de chimiothérapie chez les hommes en comparaison des femmes, en m'appuyant sur plusieurs études internationales, que j'ai analysées à partir des données source.

L'ensemble de ces résultats indique que l'intégrité du système circadien, est un facteur déterminant de l'efficacité et de la tolérance de la chimiothérapie, et ce tout au long du traitement. Il en résulte qu'une nouvelle stratégie chronothérapeutique qui préserverait ou restaurerait la fonctionnalité du système circadien pourrait améliorer à la fois l'efficacité et la tolérance de la chimiothérapie.

Afin d'optimiser les interventions thérapeutiques, il est nécessaire d'approfondir les mécanismes de la disruption circadienne liée au cancer ou à ses traitements. En particulier, les cytokines pro-inflammatoires pourraient jouer un rôle important dans cette disruption, ainsi que nous l'avions précédemment rapporté (Sephton and Spiegel 2003; Rich, Innominato et al. 2005; Antoni, Lutgendorf et al. 2006; Coogan and Wyse 2008; Dantzer, O'Connor et al. 2008; Miller, Ancoli-Israel et al. 2008; Reyes-Gibby, Wu et al. 2008; Seruga, Zhang et al. 2008; Eismann, Lush et al. 2010). L'amélioration des connaissances dans ce domaine paraît essentielle pour choisir la stratégie chronothérapeutique la mieux adaptée au patient et à sa condition médicale ainsi qu'aux objectifs de sa prise en charge.

D' une part, cette approche originale de la chronothérapie implique l'administration de schémas de chronothérapie différents selon le sexe et le système circadien du patient. Elle s'appuie sur l'enregistrement continu de plusieurs biomarqueurs du système circadien au cours du traitement, et possiblement d'études de polymorphismes des gènes de l'horloge, afin d'adapter l'administration chronothérapeutique aux modifications induites par la chimiothérapie. D'autre part, un traitement ciblant plus spécifiquement le système circadien pourrait être mis en œuvre, chez les patients dont le système circadien est altéré. Cette perspective souligne l'intérêt d'une part du développement de médicaments dits « chronobiotiques », tels que la mélatonine ou ses agonistes/antagonistes, voire les

inhibiteurs de caséine kinase ou d'autres molécules régulatrices de l'horloge moléculaire, mais aussi d'approches comportementales, dont l'exercice physique ou les thérapies interventionnelles psychiques. Ces approches font partie d'un travail collaboratif en cours impliquant plusieurs équipes américaines (Tableau IX).

Tableau IX. Essais cliniques collaboratifs évaluant le bénéfice clinique d'un ciblage pharmacologique ou comportemental du système circadien des patients cancéreux. Le critère principal concerne les symptômes.

Pathologie Cancéreuse	# de patients	Traitement	Contrôle	Paramètre clinique	Investigateurs Principales
Sein Adjuvant	426	Paroxetine (20 mg/j)	Placebo	Troubles subjectifs du sommeil	O. Palesh (Stanford, E-U) & G. Morrow (Rochester, E-U)
Sein avancé et Leucémie Myéloïde Chronique	52	Mélatonine (20 mg/j)	Aucun	Actimétrie et qualité de vie	G. Bjarnason (Toronto, Canada)
Sein adjuvant	41	Exercice physique	Soins usuels	Actimétrie et qualité de vie	G. Bjarnason & T. Petrella (Toronto, Canada)

Une étude en cours, en collaboration avec l'équipe danoise de Camilla Qvortrup et Per Pfeffer (Université d'Odense) me conduit à explorer le rôle du rythme circadien du cortisol et du sexe l'efficacité et la tolérance d'une administration chronomodulée ou conventionnelle de la capecitabine, médicament anticancéreux pris par voie orale, au cours de deux essais cliniques chez les patients atteints de cancer colorectal métastatique (Qvortrup, Yilmaz et al. 2008; Qvortrup, Jensen et al. 2010). Enfin, plusieurs médicaments, de différentes classes pharmacologiques, ciblant soit les ligands soit les récepteurs des principales cytokines pro-inflammatoires existent ou sont en développement clinique avancé. On ne connaît ni leur impact sur le système circadien ni les possibles conséquences d'un tel impact pour le contrôle tumoral, indépendamment de l'activité antitumorale directe de ces substances. Il s'agit d'une voie de recherche que j'entends poursuivre et développer.

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IX. APPENDICE

IX.1. Résultats cliniques récents de la chonothérapie des cancers colorectaux métastatiques

IX.1.1. Article # 12, Cancer, 2009

Chimiothérapie de recours par trithérapie chronomodulée intra-artérielle hépatique chez des patients atteints de cancer colorectal métastatique lourdement traité

*Bouchahda M, Adam R, Giacchetti S, Castaing D, Brezault-Bonnet C, Hauteville D, **Innominato PF**, Focan C, Machover D, Lévi F.*

La perfusion intraartérielle hépatique (IAH) de chimiothérapie expose à la fois les métastases hépatiques et le foie sain à une haute concentration de médicaments, avec un risque de toxicité hépatobiliaire spécifique. Plusieurs voies de détoxification des médicaments et plusieurs étapes de la prolifération cellulaire sont contrôlées par l'horloge circadienne moléculaire dans le foie normal, mais cette régulation circadienne est altérée pas dans les tumeurs avancées. Dans cet article, les auteurs rapportent leur expérience de la chimiothérapie chronomodulée par voie IAH comme traitement de recours chez des patients atteints de cancer colorectal métastatique lourdement traités. Cet article examine les données de tolérance et d'efficacité de 29 patients consécutifs atteints de cancer colorectal métastatique et traités par chimiothérapie chronomodulée IAH avec plusieurs médicaments anticancéreux, après échec d'une chimiothérapie standard dans le centre des auteurs. Parmi les patients, 76% avaient seulement des métastases hépatiques et 24% présentaient à la fois des métastases hépatiques et pulmonaires. Soixante-quinze pour cent des patients avaient reçu au moins 3 lignes de chimiothérapie, y compris une chimiothérapie chronomodulée par voie intraveineuse pour 59% des patients. Les patients ont reçu une médiane de 4 cycles de chimiothérapie par voie IAH (entre 1 et 9). Les toxicités non hématologiques de grade 3 ou 4 (selon l'échelle NCIC-CTC v3) les plus fréquents étaient les vomissements, la diarrhée, la douleur abdominale et la fatigue. Aucune toxicité sévère hématologique ou hépatique, ni cholangite chimique n'a été rapportée. Une réponse tumorale objective a été

observée chez 10 patients (34.5%), dont quatre ont ensuite eu une hépatectomie de type R0 ou R1. Les médianes de survie sans progression et de survie globale étaient 4.5 mois (intervalle de confiance à 95%, 2.4-6.5 mois) et 18 mois (IC95%, 5.8-30.2 mois), respectivement. La chimiothérapie chronomodulée par voie IAH a présenté une activité antitumorale, avec une bonne tolérance chez des patients lourdement traités sélectionnés. Les auteurs considèrent que cette modalité thérapeutique mérite d'être évaluée de façon prospective dans un essai clinique mené chez des patients présentant une maladie moins avancée.

Rescue Chemotherapy Using Multidrug Chronomodulated Hepatic Arterial Infusion for Patients With Heavily Pretreated Metastatic Colorectal Cancer

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BACKGROUND: Hepatic arterial infusion (HAI) chemotherapy delivers a high concentration of drugs both to liver metastases and to healthy liver with specific, limiting, hepatobiliary toxicities. Relevant detoxification and cellular proliferation pathways are controlled by the molecular circadian clock in normal liver but not in advanced tumors. In this article, the authors report their experience with chronomodulated HAI chemotherapy as rescue therapy in heavily pretreated patients who had metastatic colorectal cancer.

METHODS: Data from all consecutive patients with colorectal cancer liver metastases who received HAI with chronomodulated, multidrug chemotherapy regimens in the authors' center after failure on standard chemotherapy were reviewed for efficacy and safety. **RESULTS:** Twenty-nine patients were treated, including 76% with liver metastasis only and 24% with liver and lung metastases. Seventy-five percent of patients had received ≥ 3 chemotherapy lines, including intravenous, chronomodulated chemotherapy in 59% of patients. Patients received a median of 4 HAI courses (range, 1-9 courses). The most frequent grade (according to National Cancer Institute of Canada Common Toxicity Criteria [version 3]) 3 and 4 nonhematologic toxicities were vomiting, diarrhea, abdominal pain, and fatigue. No severe hematologic or hepatic toxicities and no chemical cholangitis were reported. An objective tumor response was observed in 10 patients (34.5%), including 4 patients who subsequently underwent R0 or R1 hepatic resection. The median progression-free survival and overall survival were 4.5 months (95% confidence limits, 2.4-6.5 months) and 18 months (95% confidence limits, 5.8-30.2 months), respectively. **CONCLUSIONS:** HAI chronomodulated chemotherapy had well tolerated activity in selected, heavily pretreated patients, and the authors believe it

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deserves to be assessed prospectively in clinical trials among patients who have less advanced disease. *Cancer* 2009;115:4990-9. © 2009 American Cancer Society.

KEY WORDS: colorectal cancer, liver metastases, hepatic arterial infusion, chronotherapy, circadian rhythms.

Metastases from colorectal cancer (CRC) are limited to the liver in 30% to 60% of patients.¹⁻³ Disease progression in the liver leads to hepatic failure and contributes to mortality among patients with predominant liver metastases.³ Hepatic resection is the only potentially curative therapeutic option for these patients.⁴ Nearly all patients who have metastatic disease and do not undergo resection will die from their disease within 5 years.^{4,5} Systemic chemotherapy is the standard of care for patients with unresectable metastatic disease. Intravenous (iv) 5-fluorouracil/leucovorin (5-FU/LV)-based dual regimens with either irinotecan or oxaliplatin have produced increased median overall survival over treatment with 5-FU/LV alone in patients with metastatic CRC.⁶⁻¹¹ When the liver is the single or prevalent disease site, hepatic arterial infusion (HAI) of cytotoxic drugs offers theoretical advantages over standard iv administration. These include the ability to deliver high drug concentrations and tumor selectivity, because the major blood supply of macroscopic tumors derives from the hepatic artery, whereas the blood supply to normal liver mostly comes from the portal circulation.¹² Original studies using fluoropyrimidine-based chemotherapy produced higher response rates with HAI compared with iv delivery but without demonstrating an unequivocal survival advantage across studies. Nevertheless, 2 meta-analyses of the 7 original trials that were conducted in 600 patients who were registered identified significant survival advantages for HAI over iv administration.^{13,14} Three additional studies were conducted,¹⁵⁻¹⁷ but only 1 trial confirmed an increased overall survival with HAI.¹⁷ However, that study used a suboptimal reference therapy with single-agent, bolus 5-FU/LV. Finally those studies did not define the exact role of HAI chemotherapy with 5-FU in the therapeutic strategy for metastatic CRC,¹⁸ and further investigations should be encouraged with more active regimens.¹⁹ Irinotecan HAI has been explored as a 5-day continuous infusion^{20,21} or as a short 30-minute infusion.^{22,23} The data from those explorations suggest that a higher rate of irinotecan conversion to its active metabolite SN-38 is achieved with

HAI delivery and that there is greater clinical activity with short-duration HAI. Oxaliplatin HAI reportedly was feasible, and its combination with iv 5-FU-LV achieved a high response rate in pretreated patients.²⁴⁻²⁹ Both the terminal half-life of and the systemic exposure to free plasma platinum were decreased by HAI of oxaliplatin, a finding that supports its lower systemic toxicity compared with iv administration.²⁷ Such liver-directed chemotherapy could benefit further from adjustment of the drug-delivery pattern to the circadian clock in healthy hepatocytes.³⁰

Normal mammalian cells contain a molecular clock that regulates the cell cycle, apoptosis, gene expression, and DNA repair.³⁰⁻³⁴ In contrast, cells in advanced tumors have lost these clock-dependent rhythms.^{35,36} The objective of chronomodulated chemotherapy is to take advantage of the circadian differences between normal cells and tumor cells by delivering high drug doses at times when normal cells are relatively protected by low proliferative or metabolic activity, whereas tumor cells maintain high metabolic and proliferative activities. In 2 randomized clinical studies, iv, chronomodulated chemotherapy with 5-FU/LV and oxaliplatin reportedly delivered higher dose intensity to improve tolerance and to increase efficacy (including overall survival in the first study) compared with the same regimen given at a constant rate of infusion.^{36,37} Nevertheless, a recent, large, randomized trial using individual dose escalation revealed a survival advantage in men but not in women who received chronotherapy.³⁸ The combination of both HAI and chronomodulated delivery of multidrug regimens could further improve the therapeutic index of cytotoxic chemotherapy in patients who have liver metastases from CRC. The peak time of chronomodulated irinotecan at 5 AM corresponded to low proportion of S-phase cells in healthy tissues and to high Bcl-2 messenger RNA expression in oral mucosa and, presumably, in other healthy tissues.³⁹ The peak time for the chronomodulated delivery of 5-FU at 4 AM corresponded to high intracellular activity of dehydropyrimidine dehydrogenase in healthy human blood cells and to low DNA synthesis in healthy target tissues,

Table 1. Hepatic Arterial Chronomodulated Chemotherapy: Study Treatment Schedule

Drug	Daily Dose, mg/m ²	Days of Treatment	Timing	Time of Peak Concentration
Irinotecan	160	1	2 AM to 8 AM	5 AM
Oxaliplatin	20	2-5	10 AM to 10 PM	4 PM
5-Fluorouracil	600	2-5	10 PM to 10 AM	4 AM

such as bone marrow, oral mucosa, and gut.³⁹⁻⁴¹ The peak time for chronomodulated oxaliplatin at 4 PM corresponded to high reduced glutathione levels and high circulating proteins in blood.^{40,42} In this article, we report our initial experience using chronomodulated HAI regimens in 29 consecutive patients with predominantly liver metastases from CRC who were referred to our institution after the failure of standard treatments.

MATERIALS AND METHODS

Study Objectives

The objective of this study was to retrospectively assess the safety and efficacy of HAI chronomodulated chemotherapy in consecutive patients who had been pretreated heavily for metastatic CRC.

Patient Selection

Data from all patients with histologically proven, metastatic CRC, isolated (or predominant) hepatic metastases, and failure on previous standard iv chemotherapy who received HAI with chronomodulated chemotherapy in our center, were collected and analyzed retrospectively.

Study Treatment

5-FU was delivered in combination with irinotecan and/or oxaliplatin through a hepatic arterial catheter connected to a multichannel, programmable pump (Mélodie, Aguetant, France) (Table 1). Courses were repeated every 21 days or more according to patient recovery from toxicities. Treatment was continued until evidence of disease progression, intolerable toxicity, or patient refusal. No intravenous chemotherapy was given simultaneously with HAI chronotherapy, except for 2 patients who were receiving concurrent intravenous cetuximab.

Assessment

Adverse events were collected and graded according to the National Cancer Institute of Canada Common Toxicity Criteria (version 3) on a standardized form before each course of chemotherapy. Blood cell counts, biochemistry, and tumor markers were obtained before each course. Tumor size was measured using computed tomography scans after every third treatment course. Response was assessed by a multidisciplinary team that included at least a radiologist, a hepatobiliary surgeon, and a medical oncologist, according to the Response Criteria in Solid Tumors. Hepatic surgery was attempted whenever the lesions were deemed resectable after HAI therapy.

Statistical Analysis

Data were descriptive and are reported as percentages \pm standard deviations or as the median and range. Time-related parameters were reported using Kaplan-Meier estimates.

RESULTS

Patients

Between March 2000 and September 2006, 29 consecutive patients met the selection criteria described previously (Table 2). All patients had predominant metastatic liver disease, 76% had exclusive hepatic metastases, and 24% had both liver metastases and small lung deposits. Greater than 50% of patients had bulky liver involvement estimated at $>50\%$ of the hepatic parenchyma. None of the 29 patients who were receiving HAI had their primary tumor left in place, and only a single patient underwent resection of the primary tumor just before receiving HAI. Most patients were heavily pretreated, including prior hepatic resection in 41% of patients. The median number of previous chemotherapy lines for metastatic disease was

Table 2. Patient Characteristics (N=29)

Characteristic	Median (Range)	No. (%)
Age, y	62.5 (35-75)	
Sex		
Men		17 (58.6)
Women		12 (41.3)
WHO performance status		
0		13 (44.8)
1		11 (37.8)
2		5 (17.9)
Primary tumor site		
Colon		24 (82.7)
Rectum		5 (17.9)
Metastatic site		
Liver only		22 (75.8)
Liver and lung		7 (27.2)
Other*		2 (6.3)
Liver metastases		
Synchronous		20 (68.9)
Metachronous		9 (31)
Estimation of liver involvement by tumor, %		
<25		4 (13.7)
25-50		7 (27.2)
>50		18 (62)
No. of previous chemotherapy lines for metastatic disease		
1		3 (10)
2		4 (13.7)
3		16 (55.1)
>3		6 (20.6)
Previous drugs		
5-fluorouracil/leucovorin		29 (100)
Oxaliplatin		29 (100)
Irinotecan		26 (89)
Cetuximab		4 (13)
Others†		7 (27)
Previous intravenous chronomodulated therapy		17 (58)
Prior HAI flat chemotherapy		1 (3)
Best response to last chemotherapy		
PR		7 (24)
SD		4 (13.7)
PD		18 (62)
NE		1 (3.1)
Previous resection of hepatic metastases		12 (41.3)
Baseline sensory neuropathy		
Total		21 (72.4)
Grade 1		7 (24.2)
Grade 2		10 (34.5)
Grade 3		4 (13.7)
Alkaline phosphatase, UI/L	276 (69-1167)	
Lactate dehydrogenase, UI/L	476 (159-3475)	
CEA, ng/mL	103.3 (1-16,080)	
CA 19-9, U/mL	119 (<2 to 11,499)	

WHO indicates World Health Organization; HAI, hepatic arterial infusion; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; CEA, carcinoembryonic antigen.

*Lymph nodes in 1 patient and a pelvic mass in 1 patient.

†Capecitabine in 3 patients; bevacizumab in 2 patients; and combined floxuridine, raltitrexed, and mitomycin C in 1 patient each.

3 (range, 1-8 chemotherapy lines) and including prior chronomodulated iv chemotherapy in 17 patients (58%) and prior constant-rate HAI chemotherapy with floxuridine in 1 patient. All patients had received previous 5-FU/LV and oxaliplatin, and 89% of patients had received previous irinotecan. All patients had progressive disease before they received HAI, including 9 patients who had an initial response (31%). Persistent sensory neuropathy after previous oxaliplatin therapy was reported at baseline in 21 patients (72%). The median interval between diagnosis of metastatic disease and onset of HAI chronotherapy was 17.7 months (25%-75% percentiles, 14.5-21.8).

Treatment

The catheter was placed in the hepatic artery through an intercostal artery in 9 patients (31%) or during laparotomy in 20 patients (68%) for whom complete resection of metastases was not possible. Twenty-four patients (83%) received triple therapy with chronomodulated irinotecan, oxaliplatin, and 5-FU. Four patients received HAI with chronomodulated irinotecan and 5-FU (2 patients with concomitant iv cetuximab). Two patients received chronomodulated 5-FU and oxaliplatin. One hundred thirty-two cycles were administered (median, 4 cycles; range, 1-9 cycles). Treatment was discontinued because of adverse events in 10 patients (34%) including catheter occlusion or thrombosis after 2 to 9 cycles in 9 patients and severe abdominal pain without evidence of thrombosis after 4 cycles in 1 patient.

Safety

There were no patient deaths because of toxicity. Relative median dose intensities were 100% for irinotecan, 103% for oxaliplatin, and 117% for 5-FU. The most frequent grade 3 or 4 nonhematologic toxicity was abdominal pain, which occurred in 13.8% of patients. Other grade 3 or 4 nonhematologic toxicities included diarrhea (10.3%) and sensory neuropathy (3.4%) (Table 3). Sensory neuropathy of all grades was reported in 21 patients (72.4%) during HAI chronomodulated chemotherapy. Nevertheless, 21 patients (72.4%) had pre-existing neuropathy at baseline (including grade 3 in 4 patients) as a result of prior oxaliplatin exposure (Table 2). The severity of neuropathy increased in 4 patients (13.8%), remained stable

Table 3. Most Common Toxicities by Patient* (n=29)

Toxicity	No. of Patients (%)	
	Grades 1-4	Grades 3 and 4
Fatigue	25 (86)	3 (10.3)
Diarrhea	24 (75)	3 (10.3)
Vomiting	19 (65.5)	0 (0)
Sensory neuropathy	21 (72.4)	1 (3)
Mucositis	14 (43.8)	0 (0)
Abdominal pain	9 (31)	4 (13.8)
Neutropenia	14 (48.2)	1 (3)
Thrombocytopenia	9 (34)	0 (0)
Anemia	20 (68)	0 (0)
ALT/AST elevation	7 (24)	0 (0)
Alkaline phosphatase elevation	1 (3)	0

ALT indicates alanine aminotransferase; AST, aspartate aminotransferase.

*Adverse events were collected and graded according to the National Cancer Institute of Canada Common Toxicity Criteria (version 3).

in 21 patients (72.4%), and improved in 6 patients (20.6%) during HAI. Neutropenia of any grade occurred in 48.2% of patients, yet it reached grade 3 in only 1 patient. No episode of febrile neutropenia or neutropenic infection was reported.

It is noteworthy that no chemical cholangitis was reported. Compared with baseline values, transient alteration of liver function tests was reported in 9 patients (34%). Thus, serum aminotransferase activity increased by 3 to 95% in 8 patients, and alkaline phosphatase activity increased by 26 to 320% in 3 patients compared with corresponding baseline values. It also is worth noting that serum aminotransferase and alkaline phosphatase levels improved during HAI in the remaining group of patients.

Efficacy

Ten patients (34.5%) experienced a partial response (PR), 8 patients (27.6%) had stable disease, and 11 patients (37.9%) had progressive disease. Two of the 11 patients who had outright progression of their disease had progressive lung metastases yet had an objective response in their hepatic lesions. Two of the responding patients with lung micrometastases demonstrated a major response in the liver and stable lung disease. The characteristics of the 10 patients who achieved a PR are listed in Table 4. All responding patients had a World Health Organization performance status of 0 or 1. Neither concurrent lung metastases nor disease progression as best response to the

Table 4. Main Characteristics and Outcome of the 10 Patients Who Had an Objective Response to Hepatic Arterial Infusion Chronomodulated Chemotherapy

Sex	Age, y	PS	Primary Tumor	Extrahepatic Metastases	Characteristics		Response to Last Intravenous Regimen	Outcomes on Chrono HAI		
					No. of Previous Chemotherapy Lines	Last Intravenous Regimen		R0/R1 Liver Resection	PFS, mo	OS, mo
Man	55	1	Colon	No	3	FOLFOX; Chrono	PR	Yes	7	35
Man	73	0	Colon	No	1	FOLFOX; Chrono	PR	Yes	9	≥77
Man	70	0	Colon	Lung	8	Irinotecan, 5-FU, and oxaliplatin; Chrono	PD	No	5	13
Man	67	0	Colon	No	3	Irinotecan, 5-FU, and oxaliplatin; Chrono	PD	No	11	18
Man	64	1	Colon	No	3	FOLFOX; Conv	PR	No	3	23
Man	88	0	Colon	No	2	FOLFOXIRI; Chrono	PD	No	8	18
Man	60	0	Rectum	No	2	FOLFOX; Chrono	PR	Yes	≥27	≥27
Woman	65	0	Colon	No	3	FOLFOXIRI; Conv	PD	No	6	8
Woman	35	1	Colon	Lung	3	FOLFOX; Conv	NE	No	2	20
Woman	54	1	Colon	No	3	FOLFIRI; Conv	PR	No	7	≥17

Chrono indicates chronomodulated; HAI, hepatic arterial infusion; PS, World Health Organization performance status; PFS, progression-free survival; OS, overall survival; FOLFOX, leucovorin, 5-fluorouracil, and oxaliplatin; PR, partial response; 5-FU, 5-fluorouracil; PD, progressive disease; Conv, conventional; FOLFOLXIRI, leucovorin, 5-fluorouracil, oxaliplatin, and irinotecan; NE, not evaluable.

previous iv regimen precluded a response to HAI chronomodulated chemotherapy. Three of the patients who attained a PR underwent further liver metastases resection (R0 in 1 patient; R1 in 2 patients) and 1 patient with stable disease also underwent an R1 resection. The number of HAI courses received before surgery by the 4 patients who eventually underwent resection ranged from 3 courses (1 patient) to 5 courses (3 patients). One patient received 2 additional courses of HAI after surgery (R1 resection; total number of HAI courses, 7). The overall resection rate was 13.7% among 29 previously unresectable patients.

Histologically, important tumor regression with minimal viable tumor remnants was observed in the patient who underwent R0 resection and in the 1 patient who underwent R1 resection. Nontumor liver parenchyma revealed minimal fibrosis in 2 patients without vascular lesions or vascular modifications (nodular hyperplasia with peliotic foci in 2 patients).

At a median follow-up of 53.5 months (range, 17.4-95.7 months), 28 patients had experienced tumor progression, and 25 patients had died. The median progression-free survival was 4.5 months (range, 2.4-6.5 months) (Fig. 1), and the median overall survival was 18 months (range 5.8-30.2 months) since the start of HAI (Fig. 2). The median overall survival of the 29 patients on HAI chronotherapy was 36 months (range, 27.2-44.7 months) after the diagnosis of metastatic disease.

DISCUSSION

The outcome of patients with metastatic CRC has improved greatly over the past 15 years as a result of the introduction of new drugs and delivery schedules⁷⁻¹¹ and of a multidisciplinary approach with more aggressive surgical strategies.^{8,36-38,43,44} It is interesting to note that the downstaging of unresectable liver metastases with modern neoadjuvant chemotherapy regimens has allowed potentially curative resection in up to 30% of patients across published series.⁴⁵ Furthermore, a recent analysis revealed that pathologic a complete response to chemotherapy is a major determinant of long-term survival and cure in patients with metastatic CRC.⁴⁴ Therefore, a pathologic CR may represent a future surrogate endpoint.

In the current study, we attempted to deliver the most aggressive treatment to persisting tumor cells

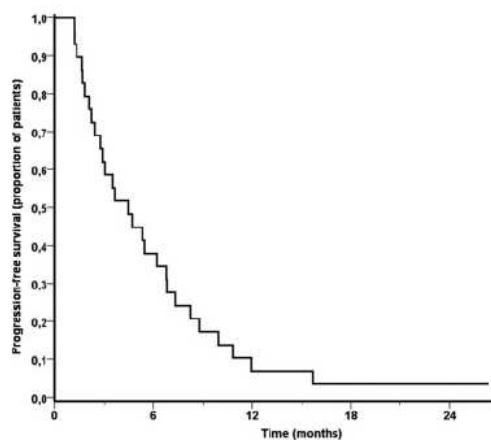


FIGURE 1. This chart illustrates progression-free survival in 29 patients who received chronomodulated hepatic arterial infusion.

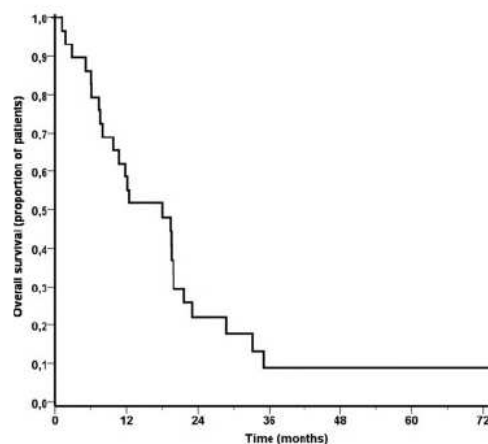


FIGURE 2. This chart illustrates the overall survival of 29 patients who received chronomodulated hepatic arterial infusion.

mainly or exclusively located in the liver by combining the most active cytotoxic drugs, a hepatic arterial route of drug administration, and chronotherapy. This therapeutic approach was given to heavily pretreated patients, including 59% who had failed not only standard therapy but also iv chronotherapy. Thus, most patients were refractory to several current standard therapeutic options.

Our cohort study demonstrated that such an aggressive HAI therapy is feasible, well tolerated, and represents an important rescue approach for selected patients. This strategy can overcome drug resistance and even permit further resection of metastasis and prolonged patient survival. Associated lung micrometastases, a frequent cause of progressive disease for patients on HAI, remained stable in 4 of 7 patients who were studied.

The specific toxicities of HAI consist of chemical hepatitis and biliary sclerosis⁴⁶⁻⁴⁸ and are believed to be the result of exposure of normal cells to high drug concentration, drug metabolism, and drug excretion. Although the hepatic arterial route of administration favors drug concentration in the tumor, healthy cells are not protected adequately, particularly the bile duct cells during drug excretion. The objective of chronotherapy is to further protect healthy cells by taking advantage of their biologic clock and exposing them to drugs when they are in a period of relative rest in their metabolic and cell division

cycles.^{30,40} Furthermore, chronotherapy theoretically should be more toxic to tumor cells because of poor circadian rhythm and greater cell cycle variability in malignant tissues compared with healthy tissues.⁴⁹

Although it has been demonstrated that HAI with irinotecan or oxaliplatin is not complicated by cholangitis,²⁰⁻²⁹ it can be anticipated in as many as 30% of patients who receive HAI floxuridine.⁴⁶⁻⁴⁸ This HAI chronotherapy regimen is well tolerated with no chemical bile duct sclerosis, whereas chronotherapy is not expected to decrease bile excretion of 5-FU metabolites. It is reasonable to hypothesize that healthy bile duct cells are protected by chronotherapy or that HAI chronotherapy favors drug metabolism into nontoxic metabolites within healthy cells. Chemotherapy-induced cholangitis carries a poor prognosis⁴⁶⁻⁴⁸; and the good locoregional tolerance demonstrated in the current study, if confirmed, in itself would make chronomodulated HAI attractive. Chemotherapy-induced hepatitis was not clinically relevant, and asymptomatic and transient elevation of liver enzymes was observed in only 28% of patients.

Oxaliplatin-based iv chemotherapy reportedly was responsible for severe sinusoidal obstruction syndrome, which has unknown clinical significance, but some possibly fatal cases have been reported.⁵⁰⁻⁵² It has been demonstrated that 5-fluorouracil and irinotecan are responsible for hepatic steatosis and nonalcoholic

steatohepatitis, which may increase postoperative morbidity.^{48,50,51} One concern is that higher intrahepatic concentrations of oxaliplatin, 5-FU, and irinotecan achieved by HAI would increase this histologic toxicity. Histopathologic examination of healthy liver in the 4 patients who underwent subsequent liver resection did not demonstrate such increased toxicity. It is interesting to note that, in the current study, oxaliplatin-related sensory neuropathy was aggravated in only 13.8% of patients but was stable in the majority of patients or even improved in 20.6%. This good tolerance may have been caused by the higher hepatic clearance of oxaliplatin administered by HAI administration versus iv administration and the subsequently lower systemic exposure shown in an animal model.⁵³ However, previous publications in humans have reported relatively small variations in systemic exposure after HAI oxaliplatin²⁷ and a 42% to 48% incidence of neuropathy with HAI oxaliplatin.^{27,29} Thus, multidrug chronomodulated HAI represents an active treatment modality for patients with metastatic CRC whose disease has become resistant to all intravenously active drugs. HAI chronotherapy may offer the greatest long-term survival benefit in patients with liver-only disease that remains unresectable or in patients who demonstrate disease progression after first-line or second-line iv chemotherapy regimens.

In conclusion, the results of the current study show that chronomodulated HAI multidrug chemotherapy is feasible and well tolerated and demonstrates antitumor activity in selected, heavily pretreated patients. Chronomodulated HAI deserves to be assessed prospectively in clinical trials that involve patients with earlier stage disease and compared with conventional approaches. Currently, novel interventional catheters have been designed especially for HAI and represent technical progress that favors the safe development of this treatment method. Thus, an ongoing European trial currently is investigating the potential of conventional or chronomodulated HAI triplet chemotherapy combined with intravenous cetuximab to increase the rate of complete resection of liver metastases from colorectal cancer.

Conflict of Interest Disclosures

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IX.1.2. Article # 13, Cancer Chemotherapy and Pharmacology, 2011

Cetuximab et chimiothérapie circadienne chronomodulée comme traitement de recours du cancer colorectal métastatique: amélioration de la tolérance, de l'efficacité et du taux de résections secondaires

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La perturbation du rythme circadien a été corrélée à des taux sériques élevés de « Transforming Growth Factor α » (TGF α), un ligand de l'« Epidermal Growth Factor Receptor » (EGFR), ainsi qu'à une moindre survie chez les patients atteints de cancer colorectal métastatique. Dans ce travail, notre hypothèse était que le blocage de l'EGFR par le cetuximab pouvait augmenter l'activité de la chronothérapie en améliorant la coordination circadienne. Tous les patients atteints de cancer colorectal métastatique adressés à notre Unité pour progression sous chimiothérapie sur une période de 30 mois ont reçu le cetuximab hebdomadaire associé à une chronothérapie bimensuelle. Cinquante-six patients ont été traités par une médiane de six cycles de chimiothérapie chronomodulée à base de fluoropyrimidines et irinotecan (61%), oxaliplatine (25%) ou les deux (14%) après une médiane de trois lignes de chimiothérapie. Aucune amplification du gène *EGFR* n'a été retrouvée par FISH dans la tumeur de 27 patients consécutifs. L'éruption acnéiforme et la diarrhée ont été les toxicités les plus fréquentes. Le taux de réponses objectives était de 32,1% . Il était corrélé positivement avec le grade d'éruption cutanée ($p = 0,025$). Aucun des patients répondeurs ne présentait une mutation du gène *KRAS* dans leur tumeur. Les médianes de survie sans progression et de survie globale atteignaient 4,6 et 13,7 mois, respectivement. Une résection macroscopiquement complète des métastases hépatiques, pulmonaires ou abdominopelviennes a pu être réalisée après réduction tumorale chez 11 patients (21%), dont 8 étaient en vie à 3 ans. Ces chiffres sont deux fois plus élevés que ceux rapportés pour la combinaison de cetuximab avec une chimiothérapie conventionnelle en première ligne, ou pour la chronothérapie seule en troisième ligne. L'ajout du cetuximab à la chronothérapie a permis un contrôle thérapeutique efficace et sûr des métastases, y compris leur résection complète, malgré les échecs antérieurs des plusieurs lignes de chimiothérapie.

Cetuximab and circadian chronomodulated chemotherapy as salvage treatment for metastatic colorectal cancer (mCRC): safety, efficacy and improved secondary surgical resectability

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Abstract

Background Circadian rhythm disruption was linked to high serum levels of Transforming Growth Factor Receptor α , an Epidermal Growth Factor Receptor (EGFR) ligand and poor survival in patients with metastatic colorectal cancer (mCRC). We hypothesized that EGFR blockade with cetuximab would enhance the activity of chronotherapy as a result of improved circadian coordination.

Methods All the patients with mCRC referred to our unit for progression on prior chemotherapy over a 30-month-period received weekly cetuximab and fortnightly chronotherapy.

Results Fifty-six patients were treated with a median of six courses of fluoropyrimidine-based chemotherapy and irinotecan (61%), oxaliplatin (25%) or both (14%) after a median of three prior regimens. We found no *EFGR*

amplification by FISH in the tumor of 27 consecutive patients. Acneiform rash and diarrhea were the most common toxicities. Objective response rate was 32.1% and positively correlated with rash grade ($p = 0.025$). None of the responders had K-Ras mutation in their tumor. Median progression-free and overall survival were 4.6 and 13.7 months, respectively. Complete macroscopic resections of metastases in liver, lung or other abdominopelvic sites were performed following tumor downstaging by the treatment regimen in 11 patients (21%), 8 of whom being alive at 3 years. These figures are twice as high as those reported for first-line combination of cetuximab with conventional chemotherapy or for third line chronotherapy.

Conclusions The addition of cetuximab to chronotherapy allowed safe and effective therapeutic control of metastases, including their complete resection, despite previous failure of several treatment regimens.

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Keywords Cetuximab · Chronotherapy · Liver resection · Metastatic colorectal cancer · Neoadjuvant chemotherapy · Circadian clocks

Introduction

The downsizing of metastatic colorectal cancer (mCRC) with safe and effective neo-adjuvant chemotherapy allows subsequent radical surgical resection of residual metastases. This medico-surgical strategy offers long-term survival to patients despite initially unresectable disease, as initially proven with circadian-based chronomodulated chemotherapy [1–3]. The incorporation of cetuximab in the neo-adjuvant chemotherapy for unresectable mCRC was recently found to be an important asset for the success of this medico-surgical strategy in previously treated patients.

Thus, addition of cetuximab to conventional chemotherapy allowed hepatic resections in 7% of the patients despite prior failure of cytotoxic chemotherapy alone [4]. Cetuximab is a chimeric monoclonal antibody directed against the extracellular domain of the epidermal growth factor receptor (EGFR). It displays activity against mCRC as a single agent [5–7]. It further enhances the efficacy of cytotoxic chemotherapy, either in first-line [7, 8], or in pretreated patients, partly because it reverts resistance to standard chemotherapy [9, 10].

Patients with mCRC can display increased circulating levels of Transforming Growth Factor α (TGF α), a natural ligand of EGFR [11, 12]. It has been previously shown that serum levels of TGF α significantly correlated with poor survival outcome as well as with circadian disruption in mCRC patients [12]. In rodents, only intracerebral infusion of TGF α or EGF, disrupted the circadian clocks in the brain. Of 40 other cytokines tested, no other produced these effects on the circadian rhythm [13, 14]. These preclinical findings emphasize the key role of TGF α /EGF/EGFR ligand receptor interaction and their downstream pathways in the regulation of the circadian clocks that determine 24-h changes in anti-cancer drug tolerability and efficacy. Circadian clocks are found in all mammalian cells and are comprised of 15 specific genes that control cell proliferation, DNA repair, apoptosis, angiogenesis, metabolism and drug detoxification [15–17]. The adjustment of chemotherapy delivery to circadian clocks—i.e. chronotherapy—improved tolerability and efficacy compared to constant rate infusion first-line treatment for mCRC [18–20]. In addition, a four- to fivefold increased tolerability was achieved with optimally versus poorly timed chronotherapy in 159 pretreated patients with colorectal or lung cancer [21]. The optimal timing ranged 1:00–4:00 a.m. for 5-fluorouracil-leucovorin (5-FU-LV) and 1:00–4:00 p.m. for oxaliplatin or carboplatin. The combination of EGFR antagonists with chronotherapy could further enhance the resectability of CRC metastases, beyond what has already been achieved with cetuximab and standard chemotherapy, as a result of improved coordination of the molecular circadian clocks that rhythmically control nearly 10% of the human transcriptome [15]. This work examines the effects of the addition of cetuximab to chronotherapy for the safe and effective therapeutic control of metastases, and even their complete resection, despite previous failure of several treatment regimens.

Patients and methods

Objective

Preliminary assessment of safety, efficacy and surgical resection rate of cetuximab associated with chronomodulated

chemotherapy in patients having failed at least one chemotherapy line.

Selection of subjects

All consecutive patients referred to our unit between March 2004 and November 2006 were given cetuximab combined with chronomodulated chemotherapy in second- or higher line of chemotherapy for metastatic disease provided they had: (1) histologically confirmed mCRC; (2) progressive disease on prior chemotherapy; (3) measurable metastases considered as non resectable with curative intent because of additional bulky disease or ill-placed lesions and/or lesions at multiple sites, (4) no overt brain metastases, (5) WHO performance status (PS) < 3.

Lack of tumor EGFR expression or presence of *K-Ras* mutation did not constitute an exclusion criterion.

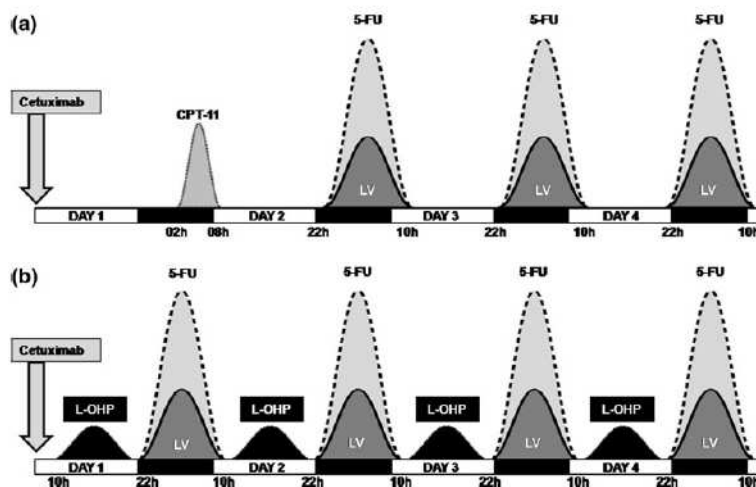
Treatment

Cetuximab was given intravenously at a loading dose of 400 mg/m² over 2 h, then weekly at a dose of 250 mg/m² over 1 h. Chronotherapy consisted of day 1 irinotecan (180 mg/m² over 6 h with peak flow rate at 5:00 a.m.), and day 2–4 5-FU-LV (900 and 400 mg/m²/day, respectively, over 12 h with peak flow rate at 4:00 a.m.) (Fig. 1a). In patients with prior clinical intolerance to irinotecan (repeat grade 3 or 4 gastro-intestinal toxicities or grade 3 asthenia), this chronotherapy regimen consisted of day 1–4 oxaliplatin (20 mg/m²/day over 12 h with peak flow rate at 4:00 p.m.) and 5-FU-LV (700 and 300 mg/m²/day, respectively), on the same chronomodulated infusion rate as reported above (Fig. 1b). Chemotherapy courses were repeated every 2 weeks. Patients with grade 3 toxicity had their treatment postponed until at least partial recovery (grade \leq 2), then resumed with a 25% dose reduction. Treatment was pursued until disease progression, grade 4 intolerable toxicity, or patient refusal.

Assessment procedures

All patients receiving at least one full course of combined cetuximab and chronomodulated chemotherapy course were assessed for toxicity and antitumor efficacy. Tumor response was determined according to RECIST criteria [23], using thoraco-abdomino-pelvic spiral CT-scan and abdomino-pelvic ultrasound (US) at baseline, then repeated every four cycles. All imaging assessments were reviewed by an independent radiologist. Adverse events were graded according to National Cancer Institute of Canada Common Toxicity Criteria (NCIC-CTC) version 3. Decision of metastases resection was made in multidisciplinary staff meeting, based upon imaging and clinical assessment,

Fig. 1 Chronotherapy schedules automatically infused with a programmable multichannel pump in outpatients every other week, following the administration of cetuximab (250 mg/m²/week) on day 1. **a** Chronomodulated irinotecan-5FU-LV: Day 1, irinotecan (CPT11, 180 mg/m²); Days 2–4, 5-fluorouracil (5-FU, 900 mg/m²/day) and leucovorin (LV, 400 mg/m²/day). **b** Chronomodulated oxaliplatin-5FU-LV: Days 1–4, 5-FU (700 mg/m²/day); LV (300 mg/m²/day) and oxaliplatin (L-OHP, 20 mg/m²/day)



consistently with prior experience [1, 2]. Progression-free survival (PFS) was calculated from inclusion to the first documentation of disease progression. Overall survival (OS) was computed from inclusion in the study until death (from any cause) or December 2009 for patients alive at the conclusion of the study.

Expression of epidermal growth factor receptor

Epidermal Growth Factor Receptor analyses were performed on 4 µm-thick paraffin-embedded sections from primary colorectal cancer and/or resected metastases. EGFR protein expression was assessed with immunohistochemistry (IHC) using EGFR Pharm Dx kit (Dako, Trappes, France) for all the patients. Only cell membrane staining with anti-EGFR antibody (clone 2-18C9) was considered to be specific. EGFR status was considered positive when $\geq 1\%$ of the tumor cells had a complete or incomplete membrane staining. Percentage of tumor cells expressing EGFR was determined and the intensity of staining was semi-quantitatively assessed as follows: 0, no staining; 1+, weak; 2+, moderate; 3+, strong. When the staining intensity was heterogeneous, the highest intensity was utilized. Hepatocytes and peripheral nerves served as positive internal controls and positive (HT-29 cell line) and negative (CAM-1 cell line) external controls were also used.

EGFR gene copy number was assessed by fluorescent in situ hybridization (FISH) using the EGFR/CEN-7 FISH Probe Mix (Dako, Trappes, France), the Texas Red-labeled DNA probe (EGFR) bound to a 196 kb segment containing the *EGFR* gene on chromosome 7q11.2 and the fluorescein-labeled DNA probe (CEN-7) bound to the centromeric

region of chromosome 7. *EGFR* amplification was assessed in the tumors from the initial consecutive 27 patients.

Determination of KRAS mutations

The KRAS mutational status was performed on available and technically adequate primary tumor and/or subsequently resected metastases from 14 responders and 2 patients with stable disease (Table 4). Genomic DNA purified from paraffin-embedded tissues was used after histological quantification of tumor tissue in each tumor sample by HES coloration. KRAS mutations located within codons 12 and 13 were screened using the allelic discrimination assay. The detection threshold of our technique was tested using dilution of DNA bearing the various KRAS mutation into normal DNA. All mutations were detectable up to dilution of 1%, except G12V up to 5% and G12S up to 10%. Each sample analysis was performed in duplicate, and wild type and mutated KRAS controls were used in each experiment.

Statistical analyses

Fisher's exact test was used to calculate the univariate correlation between EGFR expression, acneiform rash, and chronotherapy regimen with response to cetuximab. Survival curves were calculated using the Kaplan–Meier method and compared using the log rank test. Univariate analysis was performed on the following factors: gender, age, performance status, site of primary tumor, number of metastatic sites, percent liver involvement, presence of lung metastases, presence of peritoneal metastases, CEA

level, CA19.9 level, EGFR IHC status, number of prior chemotherapy lines, prior failure to fluoropyrimidines, oxaliplatin or irinotecan, prior surgery for metastases and acneiform rash. Those factors with $p < 0.20$ were tested in multivariate analyses based on the Cox model for PFS and OS. Analyses were carried out using the SPSS software (SPSS Inc, USA, version 16.0). The level of significance was set at $p = 0.05$.

Results

Patient characteristics

The baseline characteristics of the 56 consecutive patients meeting the selection criteria are presented in Table 1. The median age was 61 years (range 35–80 years), and 61% were male patients. Patients were heavily pretreated as 86% of them had received two or more previous chemotherapy regimens and 66% had undergone prior resection of metastases. Of note, 75% of the patients had already been given chemotherapy and 84% had been exposed to all three major cytotoxic drugs used for treatment of mCRC, including 5-FU, irinotecan and oxaliplatin. A total of 367 fortnightly treatment courses were administered [median 6 (range 1–22)].

Toxicity and dose intensities

Three patients displayed grade 4 allergic reactions during the first cetuximab infusion prompting discontinuation of the drug. Thus, 53 patients received at least one full course of cetuximab and chemotherapy and were fully assessable. No toxic death was encountered.

Skin toxicity, diarrhea and peripheral neuropathy were the most frequent grade 3–4 adverse events (Table 2). Pre-existing Grade 2–3 sensory neuropathy was reported for 16 patients (28%, including 21% of the patients with Grade 2 and 7% of the patients with Grade 3).

Skin reactions of any kind and any grade were observed in 94% of the patients. Acneiform rash occurred in 83% of patients and reached grade 3 in 34% of the patients. Paronychia cracking on the fingers and/or toes was observed in 19% of the patients. Forty percent of the patients displayed skin cracks, 28% dry skin (xerosis) and 24% facial erythema.

Hypomagnesaemia was grade 1 in 40% of patients, grade 2 in 7% and grade 3 in 2%. Hematological toxicity was mild with grade 4 neutropenia recorded in less than 10% of the patients, without febrile neutropenia or infection, or influence on cetuximab dosing (Table 2). More patients given irinotecan-based regimens experienced

Table 1 Patient characteristics ($N = 56$)

	No. of patients	%
Gender (M/F)	34/22	61/39
PS (WHO)		
0	37	66
1	14	25.0
2	5	9
Primary tumor		
Colon	32	57
Rectum	24	43
Number of metastatic sites		
1	15	27
2	23	41
>2	18	32
Organs involved		
Liver	43	77
Lung	38	68
Peritoneum	6	11
Other	18	32
Baseline serum CEA		
≤Normal (N)	11	20
N to 10 × N	23	41
>10 × N	22	39
Baseline serum CA19-9		
Not determined	3	5
≤Normal (N)	20	36
N to 10 × N	20	36
>10 × N	13	23
No. of prior chemotherapy lines		
1	8	14
2	12	21
≥3	33	65
Prior exposure to cytotoxic drugs		
5-Fluorouracil	56	100
Oxaliplatin	54	96
Irinotecan	49	88
All 3	47	84
Other	25	45
Modality of prior chemotherapy		
Conventional regimen only	14	25
Chronomodulated only	11	20
Both	31	55
Prior surgery		
For primary tumor	54	96
For metastases	37	66

grade 3–4 neutropenia (13/39 vs. 0/14; $p = 0.007$) and skin rash (17/39 vs. 1/14; $p = 0.039$) as compared to those receiving oxaliplatin-based regimen.

Table 1 continued

	No. of patients	%
EGFR protein expression (IHC)		
Negative	15	27
Primary tumor only	8	14
Metastasis only	3	5
Primary tumor and metastasis	4	7
1–10%	22	39
Primary tumor only	8	14
Metastasis only	3	5
Primary tumor and metastasis	11	20
>10%	19	34
Primary tumor only	9	16
Metastasis only	3	5
Primary tumor and metastasis	7	13

Table 2 Main toxicities per patient

	Grade 1	Grade 2	Grade 3	Grade 4
Hematological				
Neutropenia	10 (19.2) ^a	10 (19.2)	8 (15.4)	5 (9.6)
Thrombocytopenia	8 (14.4)	2 (3.8)	0 (0)	1 (1.9)
Anemia	11 (21.2)	8 (15.4)	3 (5.8)	0 (0)
Febrile neutropenia	NA	NA	0	0
Gastrointestinal				
Vomiting	14 (26.4)	23 (43.4)	4 (7.5)	0 (0)
Diarhea	17 (32.1)	14 (26.4)	11 (20.8)	4 (7.5)
Mucositis	14 (26.4)	21 (39.6)	5 (9.4)	2 (3.8)
Anorexia	11 (20.8)	19 (35.8)	6 (11.3)	0 (0)
Skin and nails				
Acneiform rash	8 (15.1)	18 (34.0)	18 (34.0)	0 (0)
Crack	9 (17.0)	10 (18.9)	2 (3.8)	0 (0)
Perionyxis	6 (11.3)	3 (5.7)	1 (1.9)	0 (0)
Other				
Fatigue	18 (34.0)	26 (49.1)	5 (9.4)	1 (1.9)
Peripheral neuropathy	4 (7.5)	21 (39.6) ^b	12 (22.6) ^c	0 (0)
Alopecia	12 (22.6)	24 (45.3) ^c	–	–

NA not applicable

^a Number of patients (%)^b Including 12 patients with baseline grade 2 sensory neuropathy^c Including 4 patients with baseline grade 3 sensory neuropathy^d Including 8 patients with baseline grade 2 alopecia

Median dose intensities (mg/m²/week) over the four initial courses were 250 for cetuximab (range 104–325), 69 for irinotecan (24–105), 32 for oxaliplatin (16–51) and 1,080 for 5-FU (372–1,600). Relative dose intensities were 93.0% for cetuximab, 76.7% for irinotecan, 79.4% for oxaliplatin, and 80.0% for 5-FU.

Efficacy

Among the 53 evaluable patients, 17 patients (32% [95% CI 19.4–44.6]) had an objective response to treatment (26.4% partial and 5.6% complete). Disease remained stable in 18 patients (34%) and progressed in another 18 patients (34%). With a median follow up of 56 months (range 37–69), median PFS was 4.6 months [3.3–5.9] and median overall survival (OS) was 13.7 months [8.1–19.2] (Fig. 2). The median duration of response was 11.7 months [95% CI 9.8–13.6]. Efficacy parameters were similar across the chemotherapy regimens used. There was a significant correlation between tumor response or disease control and rash occurrence and severity (Table 3). Univariate analyses showed that median PFS and OS were prolonged in patients with severe acneiform rash, without lung metastases, or with normal CA19.9 (Table 3). Multivariate analyses confirmed that acneiform rash, lung involvement, and serum level of CA19.9 were independent prognostic factors for PFS. These three factors and age were independent and statistically significant predictors for overall survival. These analyses did not identify any other prognostic factor including PS, gender, number of prior chemotherapy lines, serum CEA level or EGFR protein expression.

Surgical resection of metastases

Eleven patients underwent surgical resection of metastases. Nine of them had received 2 or more prior chemotherapy lines before neoadjuvant cetuximab-chronotherapy. Nine patients also had prior surgery for metastatic disease. Eight patients with an objective response and three patients with stable disease or minor response underwent metastases resection after a median of seven courses of cetuximab-chronotherapy (3–15). Nine patients had R0 resection in liver (4 patients), lung (3 patients), lymph nodes (1 patient) or pelvic recurrence (1 patient), while 2 patients had R1 resection in liver. Overall, the rate of patients whose metastases were macroscopically resected was 20.7% [95% CI 10–32], including liver for 11.3% of the patients. With a postsurgical median follow up of 40.8 months (range 38–55 months), the median relapse-free survival (RFS) for the 11 operated patients was 10.0 months [95% CI 4.0–15.9] with a 80 and 43% survival estimate at 3 and 5 years, respectively.

Tumor molecular predictors of response to cetuximab-chronotherapy

EGFR immunostaining and gene amplification, and KRAS status in responders

The tumor of 15 patients (27%) was EGFR negative with IHC. On the opposite, high positivity was observed in 34%

Fig. 2 Progression-free survival (PFS, *left panel*) and overall survival (OS, *right panel*) curves for the 56 patients included in the study (intent to treat, PFS, 55 events; OS, 46 events). Median PFS [95% CI], 4.6 months [3.3–5.9]; median overall survival, 13.7 months [8.1–19.2]

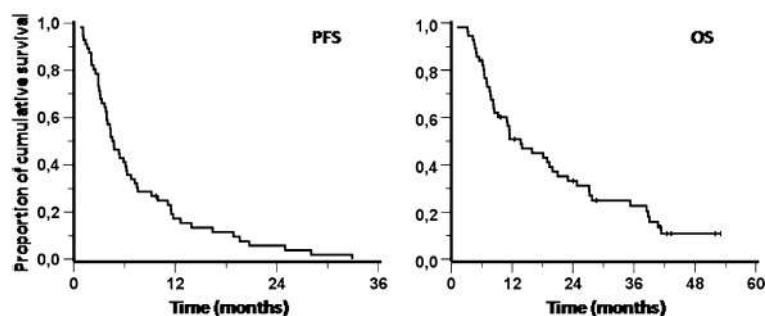


Table 3 Correlations between acneiform rash and response, progression-free survival (PFS) and overall survival (OS) in 53 patients evaluated for efficacy

Rash grade	0	1	2	3	<i>p</i> Value
Number of patients	9	8	18	18	
Objective response	0 (0) ^a	1 (12)	9 (50)	7 (39)	0.018
Disease control	3 (33)	4 (50)	12 (67)	16 (89)	0.002
PFS ^b	2.3 [1.7–3.0]	3.3 [1.4–5.3]	6.2 [5.9–6.6]	5.9 [2.9–8.9]	0.003
OS ^b	8.4 [0.0–17.0]	7.8 [4.6–11.1]	13.9 [0.0–28.9]	20.9 [11.5–30.3]	0.002

^a Number of patients (percent)

^b Median time, months, with [95% CI]

of the patients (Table 1). Tumor EGFR positive staining did not seem to significantly influence either objective response rate (EGFR+, 26%; EGFR–, 48%, $p = 0.09$), or median PFS (4.7 vs. 7.6 months, $p = 0.10$), or median overall survival (11.5 vs. 22.9 months, $p = 0.15$), even though all these efficacy endpoints appeared to be best in the patients whose tumor did not express EGFR.

No *EGFR* gene amplification was documented by FISH in the tumor specimens from any of the 27 consecutive patients tested, which led us to discontinue this assay. In these patients, objective response rate was 41%; median duration of response was 11.5 months (11 patients); median PFS was 5.9 months, and median survival was 13.7 months; the secondary resection rate was 18.5%. These figures were similar to those obtained in the 53 evaluable patients.

Of note, EGFR expression was undetectable in the tumor of 7 of the 17 responders (41%). No EGFR expression either was found in the tumor of 6 of the 11 patients with complete macroscopic surgical resections (55%).

Tumor *K-Ras* mutation was found in neither the 13 patients who achieved an objective response, nor in the 10 patients undergoing surgery, whose tumor specimens were adequate for such determinations (Table 4).

Discussion

The current study demonstrates that a combined medical–surgical strategy with curative intent can still provide meaningful clinical benefits and prolonged survival in heavily pretreated patients with mCRC. This strategy was based on both safety and antitumor efficacy of a combination of chronomodulated chemotherapy with cetuximab. Single agent cetuximab achieved an objective response in 8–12% of the patients with characteristics similar to our cohort [5, 9]. In these multicenter studies, median PFS was <2 months and median overall survival times were less than 7 months [5, 9]. The combination of cetuximab with single agent irinotecan or oxaliplatin-capecitabine produced response rates of less than 25% as second or third line chemotherapy for metastatic disease [22–24]. This antitumor efficacy further translated into median PFS of 3–5.4 months and median survival of 8.9–10.7 months [25–27]. Importantly, none of these studies reported any subsequent secondary resection of metastatic disease in such heavily pretreated patients. In our study, the combination of cetuximab with chronotherapy achieved an objective response rate of 32.1%, a median PFS of 4.6 months and a median OS of 13.7 months, in patients whose clinical and/or tumor characteristics were by far

Table 4 Survival outcomes and molecular determinants in patients with objective response and/or macroscopically complete surgical resection of metastases from colorectal cancer following salvage therapy with neo-adjuvant chronotherapy and cetuximab

Objective response	Patient ID #	Tumor site(s)	K-Ras status	Max EFGR + cells (%)	R0–R1 resection	DFS (months)	PFS (months)	Survival (months)
CR	18	T	NAv	0	Yes	6.0	11.1	11.1
		M	wt	NAv				
	26	T	NAv	10	Yes	8.3	13.9	38.8
		M	wt	NAv				
PR	38	T	NAv	1	Yes	10.6	20.7	53.1+
		M	wt	1				
	28	T	wt	0	Yes	6.0	11.8	52.0+
		M ^a	wt	NAv				
	39	T	NAv	1	Yes	1.4	6.2	39.0
		M	wt	1				
	41	M	wt	10	Yes	2.6+	9.7+	9.7+
	47	T	wt	NAv	Yes	27.3	32.9	43.3+
		M	wt	0				
	50	M	wt	50	Yes	21.9	24.9	42.5+
	9	T	wt	30	No	NAp	7.5	13.7
		M	wt	NAv				
	11	T	NAv	10	No	NAp	6.3	18.9
		M	NAv	10				
	15	T	NAv	0	No	NAp	12.6	27.7
		M	wt	0				
	19	T	wt	20	No	NAp	5.9	28.6+
		M	NAv	20				
	23	M	NAv	0	No	NAp	19.6	22.9
	25	T	wt	1	No	NAp	11.5	38.4
SD	34	T ^b	NAv	20	No	NAp	28.0	40.7
	40	T	NAv	0	No	NAp	4.6	18.1
	44	T	wt	0	No	NAp	4.3	24.7
	43	M	wt	0	Yes	10.0	16.4	41.4
	45	T	NAv	0	Yes	12.4	18.9	27.2
		M	wt	NAv				
	51	T	NAv	0	Yes	0.5	3.8	41.2+

NAp not applicable, NAv not available, T primary tumor, wt wild type

^a A mutation in K-Ras codon12 was found in a liver metastasis resected after cetuximab treatment

^b A mutation in K-Ras codon13 was found in a local relapse resected after cetuximab treatment

worse than those in the above reports [5–9, 25–27]. The efficacy of cetuximab combination with chronotherapy also compares favorably with that obtained with the chronomodulated administration of irinotecan, 5-FU-LV and oxaliplatin in 77 patients with characteristics as pejorative as in the current study [28]. Thus, a macroscopically complete surgical resection of metastases was performed in only ~ 1/3 of the patients that were successfully resected in the current study [28].

The incidence of adverse events in our heavily pretreated patients cohort was acceptable, based on a comparison with the toxicities reported for first-line cetuximab-FOLFIRI

against mCRC [8]. Thus, the incidence of severe neutropenia was 30% in our study, as compared to 28.2% in the Crystal trial. However, the incidence of non haematological toxicities was higher in our series as compared to the Crystal one for diarrhea (28.3 vs. 15.7%) and for acneiform rash (34 vs. 16.2%). We believe that the main reason for this apparent difference in non haematological toxicities relates to the fact that 86% of the patients in our study had received two prior chemotherapy lines, 84% had already been exposed to 5-FU, irinotecan and oxaliplatin, and 66% had previously undergone prior metastases resections, while no prior therapy had been offered to the patients in the Crystal

trial. In addition, the chronomodulated administration of irinotecan with peak delivery at 5 a.m. resulted in increased biotransformation into SN-38 as compared to conventional 30-min infusion at 10 a.m. [29]. The enhanced SN-38 exposure brought about by chronomodulated irinotecan could account both for increased diarrhea and increased antitumor efficacy in our series. This might also play a role in the severity of the acneiform rash although mechanisms currently remain speculative.

Indeed, an important finding in the current study is the high neoadjuvant potential activity of cetuximab's addition to chronotherapy, since 21% of the evaluable patients underwent subsequent macroscopic resection of metastases previously considered as unresectable. In this population, with very advanced disease, surgery involved multiple sites of extrahepatic metastases, consistently with the late dissemination of the disease beyond the liver itself. Despite this, the overall benefit of an aggressive medico-surgical strategy in the resected patients of our study is supported by an estimated survival rate of 43% at 5 years. This latter figure compares favorably with a 5-year survivorship of 33% in a series of 184 patients undergoing neoadjuvant chemotherapy and partial hepatectomy for liver metastases from colorectal cancer. Only 29% of the patients in this series had received prior chemotherapy [30].

The neoadjuvant potential of combining cetuximab with chemotherapy was recently shown by our team in previously treated patients [4]. An updated assessment of this latter series reveals that partial hepatectomies were performed in 5 of 45 patients on cetuximab-chronotherapy (11.1%) as compared to 5 of 98 patients on conventional delivery (5.1%), a difference that supports the apparently increased rate of complete macroscopic resections with cetuximab and chronotherapy in the current study, and merits further investigation.

Patient outcome in our cohort was correlated with the occurrence and severity of skin toxicity, as reported in most studies with cetuximab [9, 10, 23–27, 31].

A wild type *K-Ras* status has been demonstrated to predict positive response to cetuximab therapy [30, 32] and was confirmed in this cohort of patients receiving cetuximab combined with chronotherapy. Our study also confirmed that the IHC detection of EGFR protein does not predict for cetuximab efficacy [33–35]. No *EGFR* gene amplification was found in any of the tumor specimens studied. The lack of *EGFR* gene amplification was reported to predict a lack of activity of cetuximab or panitumumab, eventually combined with irinotecan-based chemotherapy [36–39]. In our study, the lack of *EGFR* amplification was consistent with its recently reported low incidence [40]. Moreover, it did not impair any of the efficacy endpoints in the patients receiving chronotherapy and cetuximab.

The improved efficacy of circadian chronomodulated chemotherapy with addition of cetuximab as compared to conventional delivery of infusion chemotherapy could stem from specific effects of cetuximab on the circadian timing system that governs chronopharmacological processes [15, 29]. Cetuximab could not only revert resistance to chemotherapy, but also prevent EGFR binding of TGF- α and restore circadian function in host cells, further improving the circadian control of malignant growth and the sensitivity to chronotherapy itself through host-mediated mechanisms [12]. In such case, the antitumor activity of cetuximab addition to chronotherapy would remain unaffected by expression of EGFR protein or *EGFR* gene amplification in tumor. Indeed, this was the case in our study, where all efficacy endpoints were highest in patients whose tumor had undetectable EGFR. Experimental and clinical data support the role of the circadian timing system as a control point in tumor progression [41, 42]. Circadian disruption has been independently associated with poor tumor response to therapy and poor survival in patients with mCRC [43], with a prospective confirmation in an international study [44]. Clinical data further support the ability of gefitinib, an inhibitor of the tyrosine-kinase domain of the EGFR, to improve circadian coordination [45]. This small molecule that impairs intracellular signaling pathways downstream of the EGFR receptor, concurrently restored a near normal rest-activity circadian rhythm and improved fatigue in advanced non-small cell lung cancer patients, despite an absence of demonstrable tumor response to therapy [45].

In conclusion, the combination of cetuximab and chronotherapy offers an effective option for salvage therapy in heavily pretreated patients. Importantly, this strategy is now demonstrated to render initially unresectable distant metastases resectable with the expectation of improved survival as part of an aggressive combined medical-surgical treatment plan.

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